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AN OSCILLOGRAPHIC STUDY OF OLFACTORY SYSTEM OF CATS*

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APART from Adrian's study (2) of the pattern of electrical activity occurring in the olfactory bulb and the pyriform lobe of the hedgehog and the lateral olfactory tract of the cat when drafts of air containing odorous substances were passed into the nasal cavity, few attempts have been made to study the olfactory regions by recording their electrical activity. The method of oscillographic recording of action potentials evoked by peripheral stimulation has proved valuable in investigations of other sensory systems and it appeared that the application of this technique to a study of the rhinencephalon might reveal more specific information concerning olfactory conduction systems. Moreover, because of certain anatomical differences unique to the olfactory pathway—this pathway does not relay in a thalamic center before reaching its primary cortex, and the afferent fibers to the primary cortex enter from the surface of the brain—it seemed quite possible that the results acquired might also provide information of general interest when contrasted with the results available from similar studies of other sensory systems.

METHODS

In a series of cats under nembutal or evipal anesthesia or after transection of the brainstem through the pons under ether, the zygoma, the temporal and the masseter muscles and the contents of the orbit were removed. The bone overlying the cerebral hemisphere was then rongoured away exposing the ventral and lateral aspects of the olfactory bulb and the ventral surface of the brain as close to the midline as possible. The animal was then placed in a supine position, the head was fixed in a holder and after section of the dura the exposed hemisphere was allowed to sag, thus giving access to the rhinencephalon. Except at the area being explored, surface drying was prevented by strips of cellophane fitted to the pia. The olfactory bulb was stimulated with single condenser discharges delivered through a transformer. The stimulating electrodes, two thin nichrome wires cemented together and completely insulated except at their tips, were inserted just beneath the surface on the lateral side of the olfactory bulb and maintained in this position by a clamp. The action potentials resulting were recorded from the surface of the rhinencephalon by a pair of ball-tipped, silver wire electrodes separated about a millimeter apart and held in position with an electrode holder. The lead electrode was always nearer the point of stimulation. The potentials were amplified with a resistance-capacity coupled differential amplifier and visualized with a cathode ray tube. The stimulus was synchronized with the sweep of the oscillograph so that a single sweep and a single shock could be produced with a manual switch. The points of recording were noted on a sketch of the exposed surface of the brain.

In another series of cats, similarly anesthetized, or after pontile brainstem transection, an area just beneath the dorsal surface of the olfactory bulb was stimulated, as described above, and the action potentials evoked were recorded with a concentric electrode oriented within the brain with the Horsley-Clarke instrument in the manner described by Ranson (18). Such electrodes were made by ensheathing an insulated copper wire with an insulated 25 gauge hypodermic needle. The cone tipped end of the copper wire, bare for 0.1 mm, pro-

* Aided by a grant from the Rockefeller Foundation.

plexiform layer superficial to this demarcation and the cell laminae deeper in position. It can be seen that the prepyriform cortex in the vicinity of the lateral olfactory tract, from the surface to a point beyond the outer cell layer, was first negative and that the remainder of this cortex laterally and dorsally was first positive (Fig 2A-B). At the cephalic end of the pyriform lobe (Fig 2C) the medullated fibers of the lateral olfactory tract spread dorsolaterally and cover the surface of the cortex. Here, due to the abruptly curved contour of the pyriform lobe, the fibrous and plexiform zone of the cortex appears of unusual depth, particularly on its ventral surface, and the reversal-point loci seemed to occur somewhere near the outer compact layer of superficial pyramids, leaving a central core of positivity. Sample records taken 2 mm apart on the same electrode tract in the cephalic portion of the pyriform cortex illustrate the inversion of the potentials. The earlier diphasic deflection seen in Fig 3a has its temporal parts in a contraposition in Fig 3b so that the temporal position occupied by the negative peak has been taken over by the positive peak and the temporal position occupied by the positive peak has been taken over by the negative peak. Furthermore, the late potential present in Fig 3a is absent in Fig 3b.

In the exploration of the interior of the brain the more dorsal records were obtained before the ventral records since the electrodes were lowered from a dorsal to a ventral position. Mention is made of this merely to eliminate from consideration any suggestion that the positivity

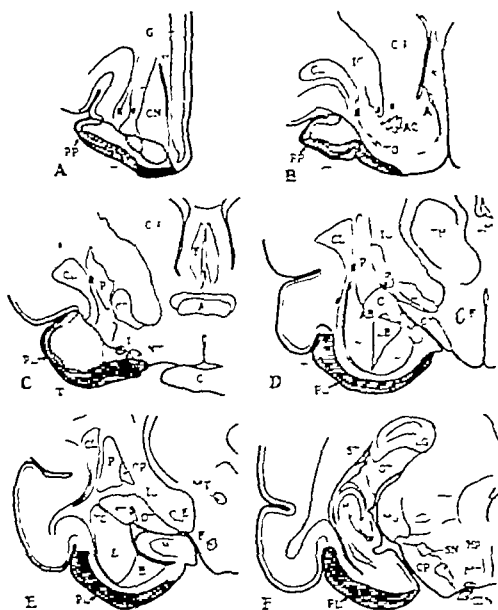


FIG 2 Six transverse sections through brain of cat at levels indicated in Fig 1, showing regions responsive to single shock stimulation of olfactory bulb. Horizontal lines indicate areas initially negative, vertical lines, areas initially positive. Peripheral extent of outer compact cell layer of olfactory cortex is indicated by broken line. Abbreviations as follows: A, nucleus accumbens, AB, longitudinal association bundle, AC, anterior commissure, B, basal amygdaloid nucleus, C, central amygdaloid nucleus, CL, claustrum, CN, caudate nucleus, CP, cerebral peduncle, E, entopeduncular nucleus, F, fornix, GG, gyrus genalis, GP, globus pallidus, H, hippocampus, HP, habenulopeduncular tract, I, intercalate amygdaloid nucleus, IC, internal capsule, LB, large celled basal amygdaloid nucleus, LG, lateral geniculate body, M, medial amygdaloid nucleus, MG, medial geniculate body, MP, mammillary peduncle, MT, mamillothalamic tract, NT, nucleus of lateral olfactory tract, OC, optic chiasma, OT, optic tract, P, putamen, PL, pyriform lobe, PP, prepyriform cortex, S, septum, SB, small celled basal amygdaloid nucleus, SM, stria medullaris, ST, stria terminalis, TC, tail of caudate nucleus, TH, thalamus, TO, olfactory tubercle, III N, third nerve.

truded for 1 mm beyond a 0.1 mm bare rim of the hypodermic needle. Systematic exploration of the base of the forebrain was undertaken and subsequently the points of recording were identified by microscopic examination of Weil-stained sections cut serially by the dry-ice freezing method of Marshall (12). Shrinkage was practically negligible with this method of preparing and sectioning brain tissue, thus permitting more accurate determination of the points where the tips of the electrodes had been when electrical activity was encountered.

RESULTS

Distribution and initial sign of recorded potential The areas excited on the ventral surface of the telencephalon by single shock stimuli of the olfactory bulb were the prepyriform cortex, the anterior olfactory lobe (20) medial to

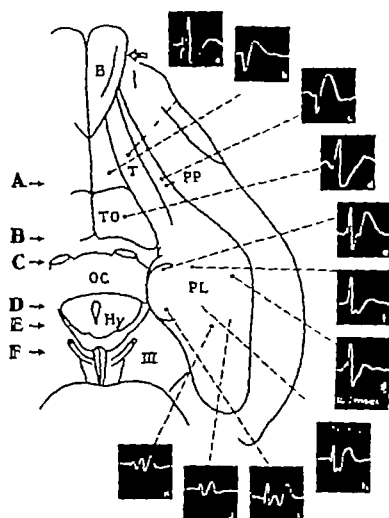


FIG 1. Ventral view of telencephalon of cat showing typical responses (a-k) evoked by single shock stimulation of olfactory bulb (open arrow at top of figure). Upward deflection in these and all subsequent records indicates negativity at "active" lead. Time in records (a-g) as in (g) and in records (h-k) as in (h). Abbreviations as follows: B, olfactory bulb, Hy, hypothalamus, OC, optic chiasma, PL, pyriform lobe, PP, prepyriform cortex, T, lateral olfactory tract, TO, olfactory tubercle, III N, third nerve. Levels A-F at left indicate plane of transverse sections shown in Fig. 2.

the prepyriform cortex, the olfactory tubercle and the pyriform lobe. Some of the more readily reproducible types of electrograms elicited from these rhinencephalic regions and the places from which they were recorded are shown in Fig. 1. Disregarding for the present their complete configurations and temporal details and considering only the sign of initial potentials, it is interesting to note that they were surface negative on the pyriform lobe, the olfactory tubercle and on that portion of the prepyriform cortex where the lateral olfactory tract is macroscopically visible. On the prepyriform cortex lateral to the lateral olfactory tract they were always surface positive while medial to the lateral olfactory tract on the surface of the anterior olfactory lobe they were sometimes negative and sometimes positive.

When explorations of the interior of the brain were made for sites responsive to olfactory bulb stimulation the same areas, the anterior olfactory lobe, the olfactory tubercle, the prepyriform cortex and the pyriform lobe, were found to be responsive. These results are summarized in six transverse sections (Fig. 2A-F) taken at the levels indicated by the small arrows A-F in Fig. 1. The regions exhibiting clear evidence of electrical activity are outlined

with horizontal and vertical lines, the horizontal lines delimiting the areas in which the initial wave forms were negative and the vertical lines delimiting areas where the initial wave forms were positive. The broken lines running through the olfactory cortex show the peripheral extent of the outer compact cell layer of this cortex and set the boundaries between the

observed in the diagonal band (Fig 2B-C), the septum (Fig 2B-C) or the hippocampus (Fig 2E-F) The amygdaloid nuclei (Fig 2C-E) were likewise silent except perhaps for a small part of the cephalic end of the anterior amygdaloid area (Fig 2C) In two cats the electrodes were oriented higher than intended and positive potentials were picked up as follows in and near the internal capsule (Fig 2A), in the external capsule between the claustrum and the putamen, in the internal capsule, and in the caudate nucleus (Fig. 2B), in the putamen or the external capsule (Fig 2C) and along the claustrum, the external capsule, the putamen, the globus pallidus and the entopeduncular nucleus (Fig 2D)

Characteristics of potentials recorded in prepyriform cortex and anterior olfactory lobe The potential records from the surface of the prepyriform cortex in the region where this cortex is covered by the macroscopically visible lateral olfactory tract exhibited (Fig 1a) a fast negative spike immediately succeeded by an initially negative, diphasic spike-like wave of greater amplitude Prolonged from the positive trough of this latter deflection was a slow low negative wave which appeared as though it might have represented an after potential The attainments of these peaks after stimulation ranged from 1.8 msec to 2.2 msec for the fast negative spike and from 6 to 8 msec for the negative spike-like wave A slight variation from this is seen (Fig 4) in a series of potentials secured from a point on the lateral olfactory tract near the caudal end of the prepyriform cortex In these records the initial negative notch was small and the subsequent spike-like wave was not diphasic but instead there was an additional negative wave appended to its descending phase Interestingly, the amplitude of this added wave could be gradually augmented (Fig 4a-f) by progressively increasing the strength of the stimulus When two stimuli were given (Fig 4g) the additional negative wave disappeared on the second stimulus while the spike-like wave was facilitated and the fast negative spike was conducted unaltered

The fast negative spike was usually diminutive and to pick it up it was necessary for the electrode to be on or near the lateral olfactory tract Commonly, as in Fig 4, it was no more than a negative notch prefixed to the greater discharge that supervened. This was especially true in the recordings obtained with an electrode oriented within the brain The potentials pictured in Fig 5a were procured from the tip of an electrode in the lateral

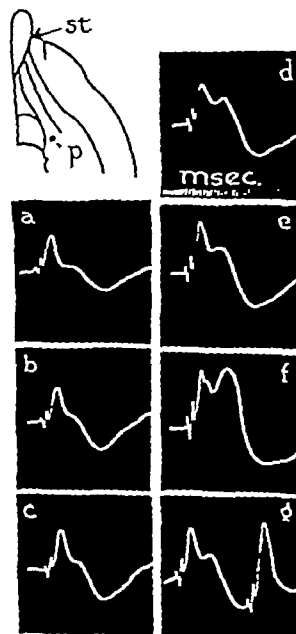


FIG 4 Potentials recorded on prepyriform cortex showing fast negative notch, the increasing amplitude of late negative wave with increasing strength of stimulation (a-f) and effect of two stimuli (g) In inset (st) indicates point of stimulation and (p) point of recording

may have been due to injury such as has been reported (6) when electrode tracts have been retraced. Also it is to be understood that with the electrodes employed the assigning of limits between the areas of positivity and negativity are at best only approximations, to be more certain of the points of reversal microelectrodes would have had to be used.

As the pyriform lobe widens posteriorly the areas of positivity greatly diminished and at levels through the middle of the amygdala (Fig 2D) these areas were confined to those positions where the pyriform cortex joins the neocortex dorsolaterally and to the medial edge of the pyriform lobe where the thinned cortex has been known as the cortical amygdaloid nucleus. In this latter region the initial potentials were not exclusively positive and some

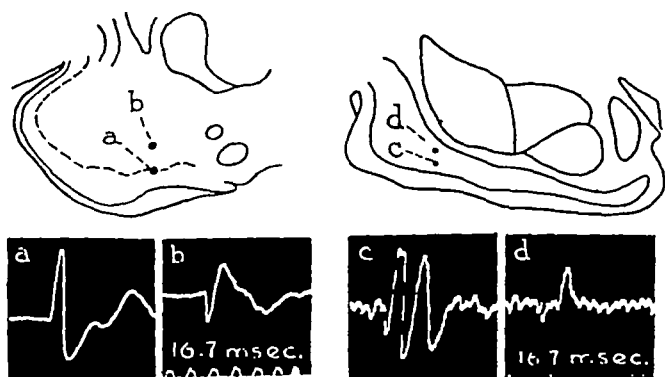


FIG 3 Potentials recorded at different depths and levels of pyriform cortex showing inversion that takes place in deeper cephalic portion of cortex (b) and simple negative phase in deeper caudal portion of cortex (d)

of the primary wave forms were negative. More caudally within the pyriform cortex (Fig 2E-F) no reversal of the initial sign occurred and the first signal of the activity set off was always a negative wave. This is illustrated with two electrograms derived 1 mm. apart on the same electrode tract in Fig 3c-d. Two negative potentials each succeeded by a positive phase are seen in the record from the point nearest the surface (Fig 3c). In the record 1 mm. deeper in the cortex (Fig 3d) the first negative wave and the positive phases are absent, the solitary negative wave present has its onset at the same time interval after stimulation as the onset of the second negative wave in Fig 3c.

On and for a short distance from the surface of the anterior olfactory lobe (Fig 2A) the first components of the responses were sometimes negative and sometimes positive. More dorsally a field of positivity extended into the underside of the rostral end of the head of the caudate nucleus and the adjacent ventral end of the internal capsule. The anterior and the lateral portions of the olfactory tubercle were also excitable but here only negative potentials were recorded (Fig 2B). No indications of electrical activity were

Olfactory tubercle The electrical activity evoked on the exterior of the olfactory tubercle (Fig 1d) was expressed by a negative potential having two peaks, the average latencies of which, as determined from the records derived from different points on the tubercles of 12 cats, were found to be 6 and 11 msec respectively following stimulation. The summits of the two peaks were not always of equal eminence like those shown in Fig 6a and there was a tendency at times for one or the other of the peaks to be more prominent. Two samples of these variations are given in Fig 1d where the first peak appeared as a mere indentation on the rising phase of the negative potential and was completely overshadowed by the second peak, and in Fig 9a, where the apex of the first peak was at a higher elevation than that of the second peak.

When two stimuli were delivered the first peak was sometimes enhanced by the second stimulus while the second peak was always erased from the record. This is demonstrated in Fig 6a and Fig 9b-e. In the latter figure the second stimulus followed the conditioning stimulus at intervals of 21, 25, 40 and 61 msec. Measuring from the point of the second shock artifact to the peak of the wave it is obvious that facilitation was greater when the second stimulus fell within the period of return from positivity to the base line (Fig 9c-d).

Some information concerning the course of impulses to the olfactory tubercle was revealed by experiments in which cuts were made either just cephalic to the level of the tubercle across the anterior olfactory lobe or across the lateral olfactory tract. The positions of the cuts and the records before and after are shown in Fig 6. The configuration of the normal potential is seen in Fig 6a. Both peaks of the potential persisted following cut 1 in the anterior olfactory lobe (Fig 6b) though in this instance the second peak became more conspicuous, however, after section of the lateral olfactory tract (cut 2) just rostral to the tubercle the entire potential was abolished (Fig 6c). The responsive region, as determined with the probing electrode, was confined to the anterior portion and the posterolateral part of the olfactory tubercle, and its depth (Fig 2B) was considerably less than the depth of the active area of the prepyriform cortex. Within this area of the tubercle negative potentials were obtained which showed only rarely the two peaks that were seen in the surface recordings. The usual sign of electrical activity was a simple negative wave that peaked at 8 msec and occasionally at 6 msec.

Pyriform lobe The records procured from the surface of the pyriform lobe were not always similar in form but they did have sufficient features in common to recognize their pattern and to allow certain generalizations. They

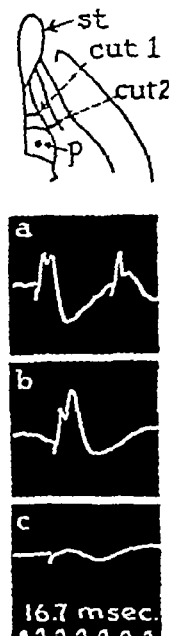


FIG 6
Potentials recorded at point (p) on olfactory tubercle, (a) shows effect of two stimuli, (b) record after cut 1 and (c) record after cut 2

olfactory tract and the adjacent anterior olfactory nucleus immediately behind the olfactory bulb at that level of the olfactory crus where the anterior olfactory nucleus forms a ring of gray about the ventricle. Here the fast negative notch and the negative spike-like wave are easily recognized. The subsequent behavior of these two components under the influence of anoxia are illustrated (Fig 5b-e) at successive stages after stabbing the heart. The record (Fig 5b) was taken when the animal stopped breathing and the final

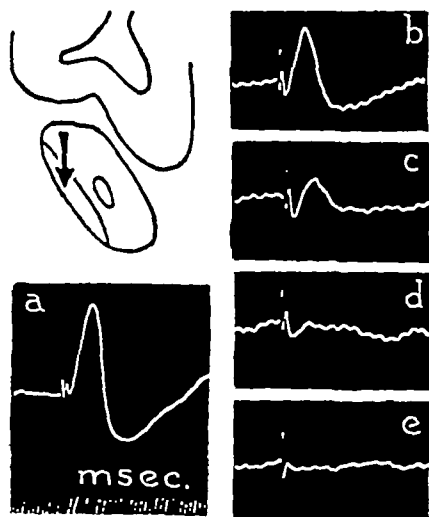


FIG 5 Potentials recorded near cephalic end of olfactory crus with recording electrode in lateral olfactory tract and adjacent anterior olfactory nucleus, showing effects of anoxia on fast negative notch and negative spike-like wave. The record (a) was obtained before heart was stabbed, record (b) was taken when animal stopped breathing following stabbing heart and record (e) was taken two minutes later.

record (Fig 5e) was taken two minutes later. The susceptibility of the negative spike-like wave and the relative resistance of the fast negative notch to anoxia are apparent, the former ebbed rapidly and vanished while the latter persisted longer and remained unaltered until it disappeared. In explorations through the olfactory crus and the prepyriform region the fast negative notch no longer appeared when the electrode was moved from the immediate environment of the lateral olfactory tract. The field in which it was present was not as extensive as the area outlined with horizontal lines in Fig 2A-B, from which the negative spike-like wave was derived, for records were obtained of this wave without the first negative notch.

The initial potentials encountered in the remainder of the responsive area, shaded with vertical lines (Fig 2A-B), were positive. On and in the lateral part of the prepyriform cortex the inversion tended to be diphasic, presenting the configuration (Fig 1c) of a positive wave succeeded by a negative wave of longer duration and oftentimes of greater amplitude. The positive phase peaked at 5 or 6 msec, and from the negative peak of the deflection, which occurred at 21 msec, the excursion leveled off about 50 msec after the shock stimulus had been delivered. A somewhat similar type of response (Fig 1b) was met with on and near the surface of the anterior olfactory lobe, although, as was previously noted, this was not always the case, for initial negative potentials were also seen in this region. In the deeper portions of the area indicated as positive (Fig 2A-B), away from the surfaces of the prepyriform cortex and the anterior olfactory lobe, the elevation that followed the positive phase subsided until only simple monophasic waves with positive peaks at 6 msec were observed.

strength of stimulus and all that was registered from the point on the pyriform lobe was the late wave (Fig 7c) preceded by a positive depression that fell in the same position as the descending phase of the second negative wave in Fig 7a. Thus if the deflections derived following section of the tract (Fig 7c) were added to the deflections obtained with the weaker stimulus (Fig 7b) the composite configuration that could be made would approach that of the original seen in Fig 7a. When the cut across the olfactory tract was subsequently deepened and the olfactory bulb stimulated, all the waves disappeared (Fig 7d), but they reappeared (Fig 7e) when the stimulating electrode was placed in the cut and the shock was given. This latter record was similar in form to that of the first, although the latencies of its respective peaks (Fig 7e) when compared with the latencies of the peaks in the first record (Fig 7a) were decreased from 10 msec to 8.3 msec, from 33.2 msec to 25.9 msec and from 87 msec to 66 msec. Stimulation of a point on the olfactory tract behind the cut gave a diphasic response (Fig 7e) similar to that which ensued when the olfactory bulb was stimulated with the weaker stimulus (Fig 7b) before the tract was cut.

At this time it should be pointed out that the late wave which persisted following section of the lateral olfactory tract could be obtained from all parts of the pyriform lobe explored. Similarly on the prepyriform cortex the late wave that could be added to the record by increasing the strength of the stimulus (Fig 4f) could be elicited after the tract fibers had been cut. The transformation in the pattern of electrical activity on the pyriform lobe produced by two stimuli is illustrated in Fig 8a, b, d and e with recordings from the respective points shown in the inset. In each case the first stimulus to the olfactory bulb resulted in two negative potentials with well separated peaks. The second stimulus altered these by eliminating the second potential and markedly increasing the amplitude of the first. The average latency of the solitary peak following the second stimulus was 14.7 msec as compared with the average latency of 10.5 msec for the first peak after the first stimulus.

The record (Fig 8c) differs from the others of this figure and is of special interest for here the two stimuli were not given to the olfactory bulb but were applied to a point on the lateral olfactory tract just caudal to a cut that severed the rostral end of the tract from the olfactory bulb. The second

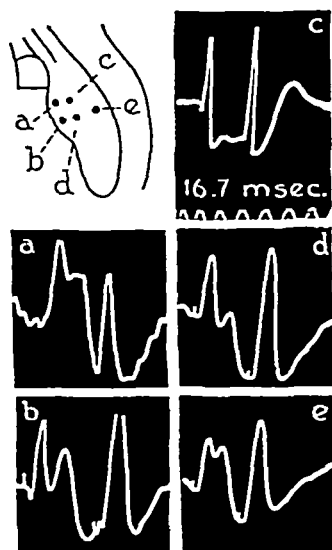


FIG 8 Potentials recorded on pyriform lobe, showing transformation following second stimulus. The first negative phase was enhanced and second negative phase was eliminated. Records (a, b, d and e) produced by stimulating olfactory bulb, record (c) produced by stimulating olfactory tract caudal to cut that severed tract from olfactory bulb.

were invariably initially surface negative (Fig 1e-k) and possessed at least two negative peaks, usually well separated. The primary negative peak arrived on a spike-like wave 8 to 10 msec after stimulation and its elevation was always more prominent than that of the negative peak on the second wave which was attained 20 to 35 msec after the shock stimulus. A third and still later negative wave could be annexed to the record by increasing the strength of the stimulus given the olfactory bulb. When the first wave was diphasic the amplitude of the second wave was so diminished that its negatively directed peak barely reached the zero line. The addition then of the third and final negative wave gave that portion of the record between the first and last peaks the appearance of the letter W (Fig 1f, h and i and Fig 7d). Considered otherwise, the resulting electrogram might be looked upon as consisting of two negative waves with a positive wave succeeding the first negative wave and a positive wave preceding the last negative wave. A variant of this is seen in Fig 1e where the final negative wave arose from

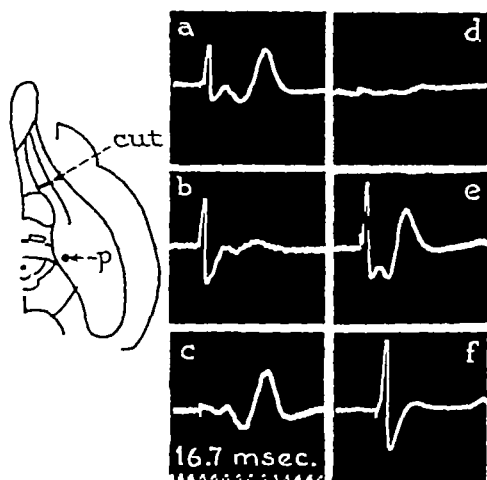


FIG 7 Records (a-d) obtained at point (p) on pyriform lobe following single shock stimuli of the olfactory bulb (a) control, (b) when strength of stimulus was reduced, (c) after olfactory tract had been cut (inset), (d) after cut had been deepened to sever prepyriform cortex. Records (e-f) taken at same point (p) (e) stimulating electrode placed in deepened cut, (f) stimulating electrode placed on olfactory tract behind cut.

the zero line after an almost imperceptible precursory positive interval. The amplitude of the second wave was increased and the latency of the second peak was decreased (Fig 8a, b, d and e) when the first wave was not diphasic. Caudally on the pyriform lobe the responses were small and exhibited two or three negative potentials (Fig 1j-k) depending on the strength of the stimulus employed.

Some light was shed on the possible origin of the potentials in these records by using different strengths of stimuli and stimulating various points in this conduction system before and after sections between the olfactory bulb and the pyriform cortex. The results of one of these experiments are summarized in Fig 7. A shock stimulus was delivered to the lateral side of the olfactory bulb and the three waves (Fig 7a) were picked up at the point p (inset Fig 7) on the pyriform lobe. This procedure

was repeated with the strength of the stimulus reduced and the resulting record (Fig 7b) exhibited the first diphasic wave and only a slight indication of the second negative wave. A superficial cut (inset Fig 7) was then made across the lateral olfactory tract just deep enough to sever the tract fibers. The olfactory bulb was then stimulated with the greater

from subjacent thalamic centers by thalamo-cortical fibers which first penetrate the deeper cortical layers. When a recording is made at the end of such a neural mechanism, *i.e.*, on the surface of the cortex, the primary wave is positive, which agrees with the idea that impulses coming towards an electrode produce positive signs.

The olfactory cortex is different. Its afferent connections do not reach the cortex from a deeper lying thalamic center but rather they come from the anteriorly placed olfactory bulb, and approach this cortex from its surface in the externally situated lateral olfactory tract. The collaterals and terminals of the lateral olfactory tract fibers end in the plexiform layer of the olfactory cortex (15, 17) where they synapse with dendrites principally from the layer of superficial pyramids. A few dendritic expansions from the layer of deeper pyramids also reach this region. Thus the receptive field of the primary olfactory cortex is located in the plexiform layer. Marshall, Talbot and Ades (14) have given evidence to indicate that certain negative reactions might take place through the plexiform layer of the cortex. They have suggested that besides those negative waves considered as indicating corticofugal and descending intracortical activity (impulses going away from the surface electrode) it is possible that activity propagated tangentially in the cortex may also exhibit negative waves. If activity so directed does give rise to a negative potential then the primary wave most likely to be expected on the surface of the olfactory cortex would be negative, for the organization in this cortex resulting from the peripheral position and linear distribution of the lateral olfactory tract provides an ideal anatomical arrangement for the propagation of activity in a tangential direction.

That the superficial location of the lateral olfactory tract may be a determining factor in the form of the potential is further suggested by the fact that the potentials were inverted on that portion of the prepyriform cortex lateral to the macroscopically visible olfactory tract (Fig 1c and 2A-B). Reversal in sign was also seen when the electrode was moved into the deeper layers of the prepyriform cortex and the deeper layers of the cephalic portion of the pyriform cortex (Fig 2C and Fig 3a-b). This would seem to follow from changing the position of the recording electrode with reference to the direction of propagated activity. The areas in which this reversal occurred within the cortex diminished rapidly posteriorly until initially positive potentials were present only in the medial and lateral positions shown in Fig 2D. Caudally (Fig 2E-F) this reversal in sign did not take place. Here the first wave seen in the intracortical record (Fig 3c) nearest the surface was absent in the intracortical record obtained in the deeper layers (Fig 3d) and only the later negative phase was present.

In considering the component features of the records we have been inclined toward the opinion that the fast negative spike (Fig 1a) and the fast negative notch (Fig 4 and 5) obtained from the lateral olfactory tract or from the prepyriform cortex in the immediate vicinity of the tract represented the discharge of olfactory tract axones, and that the negative spike-like wave which followed signaled the initial activity in the cortex. Com-

stimulus augmented considerably the spike-like wave that followed it demonstrating that this facilitation did not require the intermediation of the olfactory bulb

The effect of varying the interval between the two stimuli is demonstrated in Fig 9, rows B and C, taken from the respective points B and C shown in the inset. The intervals between shocks in the Row B (Fig 9f-j) were 13, 29, 42, 50 and 67 msec and for row C (Fig 9k-p) in which a slower sweep was employed they were 25, 50, 75, 116, 149 and 190 msec. The alterations in the forms and the increase in the amplitudes of the negative deflections that followed the second stimuli are apparent upon inspection. Facilitation was greater when the second stimulus happened during the period of return from positivity to the base line (Fig 9g-h and k-l). At the longer time interval of 190 msec (Fig 9p) the elevation of the negative discharge was no longer increased by the second stimulus. The positive phase was particularly prominent in the record taken from the point C and it was especially enhanced when the second stimulus occurred a short interval after the zero line had been reached (Fig 9m-o)

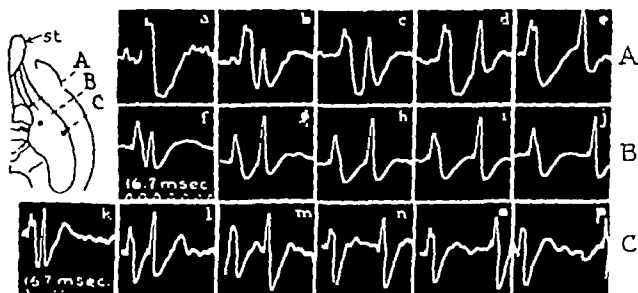


FIG 9 Record showing effect of varying interval between two stimuli given olfactory bulb. Row A, records from point (A) on olfactory tubercle. Rows (B) and (C) records from points (B) and (C) on pyriform lobe. Time intervals between stimuli: records (b-e) 21, 25, 40 and 61 msec; records (f-j) 13, 29, 42, 50 and 67 msec; records (k-p) 25, 50, 75, 116, 149 and 190 msec.

DISCUSSION

It is worthy of note that the initial response on the pyriform lobe and part of the prepyriform cortex induced by activating the afferent olfactory pathway was a negative wave, since the initial response on the surfaces of other sensory cortices comparably activated, the somatic sensory (13), the auditory (1), and the visual (5, 7), is a positive wave. One of the considerations that has played a part in interpreting the phases of cerebral action potentials has been the direction of the propagated discharge relative to the position of the recording electrode. Thus investigators (14) have accounted for these phases, partly at least, on the assumption that impulses approaching an electrode produce positive signs and impulses leaving an electrode produce negative signs. In the somatic sensory, the auditory and the visual systems activity initiated in the cortex by peripheral stimulation is relayed

the time interval between the two stimuli reached 190 msec (Fig 9p) The elimination of the second negative peak by the second stimulus (Fig 8, 9 and 4g) can be considered in several ways It may mean that the neural elements responsible for the two peaks have different recovery periods, in which case the refractory period for the neurons contributing to the second peak is rather long, since this peak failed to show (Fig 9p) when the second stimulus was delivered 190 msec after the first stimulus On the other hand the elimination of the second peak may mean that the activity in the cortex becomes synchronized and that the same elements, that previously fired separately causing two peaks, discharge in unison giving rise to a single wave of greater amplitude Theoretical analyses of the probable way in which the cortex operates, as deduced from its finer anatomy, can be found in the Golgi studies of Lorente de Nó (11) on the entorhinal area and O'Leary (15) on the primary olfactory cortex These investigators have directed attention to the intracortical neuron chains and to the abundance and systematic distribution of the cells of short axis cylinder in these chains One of the functions they have suggested for these cells is that of synchronizing cortical activity and bringing about the participation of more and more elements in this activity

The behavior of the late negative wave which required stronger stimulation of the olfactory bulb (Fig 7b) gave information concerning an intracortical conduction system within the prepyriform and pyriform cortices This wave was not eliminated by section of the lateral olfactory tract (Fig 7c) and to abolish it (Fig 7d) the section had to be deep enough to cut through the prepyriform cortex Stimulating within the cut caused the wave to reappear (Fig 7e) but stimulating a point on the olfactory tract caudal to the cut failed to elicit it (Fig 7f) These observations indicate that a trans-cortical connection must exist between the prepyriform and the pyriform cortices This late wave could be obtained from all parts of the pyriform lobe explored and on the prepyriform cortex a similar late wave, likewise dependent on stronger stimulation of the olfactory bulb (Fig 4f), could be derived after rostral tract section This combination of evidence gives some insight into the probable sequence of events that transpire in the olfactory cortex when this system is stimulated from the periphery It is clear that the olfactory tract initiates activity in the prepyriform and pyriform cortex In turn the excitation set up in the prepyriform cortex may reactivate the more caudal prepyriform cortex and the pyriform cortex under normal physiological conditions as it appears to have done in these experiments

The important feature of the responses on the olfactory tubercle was that they were recorded on the tubercle In a contemporary independent investigation of the olfactory brain by this same method, the abstract of which has just appeared, Rose and Woolsey (19) observed no potentials on the tubercle This is interesting because in the ordinary laboratory animals there has been no convincing anatomical evidence to indicate that this structure belongs to the olfactory system Ramón y Cajal (16) was not able to

parison of the behavior of these two components when a second stimulus was given (Fig 4g) and under the influence of anoxia (Fig 5b-e) favors this interpretation. The negative notch was not increased in amplitude by a second stimulus and when the heart was stabbed it persisted longer and then disappeared suddenly which would seem to indicate that this discharge was pre-synaptic in character and that tract fibers were involved. Under the same conditions the negative spike-like wave was increased in amplitude by a second stimulus and it was depressed in rapid stages and disappeared sooner when the heart was stabbed, which would seem to indicate that synapses were involved and that this discharge was post-synaptic in character.

On the other hand, in view of the anatomy of the olfactory bulb (17), it is conceivable that both the negative notch and the negative spike-like wave may have represented activity in the lateral olfactory tract and still have been pre-synaptic and post-synaptic respectively. In this interpretation we must assume that the shock stimulus delivered just beneath the surface of the olfactory bulb stimulated only a few axones of mitral cells giving rise to the pre-synaptic negative notch and that the greater post-synaptic negative wave resulted from exciting indirectly a greater number of mitral cells by stimulating the entering olfactory nerve fibers and the granule cells which associate the olfactory nerve fibers with adjacent and nearby mitral cells. It could then be considered that the neural mechanism responsible for the enhancement of the negative spike-like wave seen on the second stimulus resides in the olfactory bulb. An argument against this is the fact that this wave could be increased in amplitude when the olfactory tract was stimulated with two successive shocks, after the tract had been completely severed from the olfactory bulb (Fig 8c) and there was no possibility for this latter structure to contribute to the augmentation of the potential. For this reason we have favored the interpretation that initial activity in the cortex itself caused the spike-like wave since it seemed more reasonable to assume that its enhancement would require the intermediation of some synaptic mechanism and that tract fibers by themselves would be quite unlikely to exhibit such facilitation.

In the records from the prepyriform and the pyriform cortex, altered by a conditioning stimulus (Fig 4g, 8 and 9), we have estimated the increased response of the first wave by measuring the elevation of its peak above the background from which the deflection arises rather than the elevation it attains above the zero line. If this method of measurement gives an index of the quantity of elements participating in the discharge, then it appears that the most favorable time for the second stimulus to exploit the background of excitement created by the conditioning stimulus is during the period of the return from positivity to the zero line (Fig 9g, h, k and l). When the second stimulus occurred earlier, *i. e.*, when the background was going positive, the increased amplitude of the negative wave was negligible (Fig 9f). The condition brought about by the first stimulus endured for some time after the zero line had been reached (Fig 9i, j, m and n) but it wore off when

tained in only two cats in which the electrodes were oriented higher than intended and further explorations were not made in other animals. It is interesting, though, that support for connections between the olfactory system and the striatum can be found in the literature. The term "olfactostriatal area" has been employed in comparative neurology (3). Also Goldstein and Riese (10) have given evidence for considering that a part of the pallidum is related in some way to the olfactory region. In a four year old child with a congenital defect of the olfactory system they found a well developed striatum but a lack of myelinization in the anterior and medial sections of the medial segment of the globus pallidus.

SUMMARY

Following single shock stimulation of the olfactory bulb in the cat, potentials were recorded on and in the prepyriform cortex, the anterior olfactory lobe, the olfactory tubercle and the pyriform lobe. No responses were obtained in the septum, the diagonal band, the amygdaloid nuclei and the hippocampus.

The initial responses were surface negative on the prepyriform cortex covered by the macroscopically visible olfactory tract, on the olfactory tubercle and on the pyriform lobe. Laterally on the prepyriform cortex the initial responses were positive and on the anterior olfactory lobe they were sometimes negative and sometimes positive. These findings were considered of interest since other sensory cortices exhibit an initially positive potential following peripheral stimulation. The different sign of response on the olfactory cortex was attributed to the different orientation of discharging elements in this cortex resulting from the surface position and linear distribution of its afferent fibers.

Deeper in the prepyriform cortex and in the cephalic portion of the pyriform cortex the potentials were inverted and the initial response was positive. Caudally in the pyriform cortex the deeper intracortical records did not show this reversal in sign. Here the first wave was absent and only a later negative phase was present.

In the immediate vicinity of the lateral olfactory tract on the prepyriform cortex a fast negative spike or notch preceded the first negative wave. This spike or notch was conducted unattenuated after a conditioning stimulus and was relatively resistant to anoxia. Indications are that it was due to impulses in olfactory tract axones.

Usually the records from the prepyriform cortex had a single negative potential, whereas the records from the olfactory tubercle and the pyriform lobe had two negative potentials. In all these regions the first negative potential could be enhanced and the second negative potential eliminated by a second stimulus.

The presence of transcortical connections through the prepyriform cortex to the pyriform cortex were indicated by a later negative wave which persisted after rostral section of the olfactory tract and was dependent on stronger stimulation of the olfactory bulb.

decide in Golgi preparations whether any olfactory fibers reached the plexiform layer of the tubercle and after extirpation of the olfactory bulb he could find no fibers degenerating to the tubercle in Marchi material. This latter method of necessity would not show unmyelinated terminals. Beccari (4) questioned the passage of olfactory tract fibers to the tubercle except possibly rostrally. The best demonstrations of the relation of the tubercle to the olfactory apparatus have been made in unusual mammals. After extirpation of the olfactory bulb in the marsupial, *Perameles nasuta*, Elliot Smith (9) has shown by the Marchi method a degeneration of olfactory tract fibers over the lateral half of the tubercle. On the gross brain of the aardvark (*Orycteropus aethiopicus*), an African edentate that has an unusually well developed olfactory system, he found macroscopic strands of fibers that sprang from the olfactory tract and spread over the olfactory tubercle. In the brain of a large ant eater (*Myrmecophaga jubata*), a South American edentate, medullated olfactory tract fibers can be seen covering most of the surface of the olfactory tubercle (Fox, unpublished). Undoubtedly, in some forms at least, this structure has some function in the olfactory system.

In the present experiments in which appropriate cuts were made (Fig 6b-c) indications are that impulses reach the olfactory tubercle by way of the lateral olfactory tract. On and in the tubercle initially negative potentials were recorded, in the tubercle it was found that it was principally the anterior and lateral areas that were responsive. The surface recordings with two negative peaks could be altered by a second stimulus (Fig 9b-e) in the same manner as could the records on the prepyriform and pyriform cortices. The discussion of this reaction given above could be applied equally well here and does not need to be repeated.

No potentials were recorded in the septum, the diagonal band, the amygdaloid nuclei and the hippocampus. Whether this means that these areas have no connections with the olfactory systems or that they are connected only after a long series of synapses and the method of study employed was inadequate, we hesitate to say. The fact does remain, however, that no evidence was forthcoming from the present experiments to indicate that they do participate in olfaction. The caudal superior portion of the pyriform lobe, designated the "écorce temporale postérieure ou supérieure" by Ramón y Cajal (17) and demonstrated by him to be the source of most of the afferents to the hippocampus, was not explored. If potentials can be obtained on this cortex after stimulation of the olfactory bulb it might be anticipated that they would be small, since the potentials derived caudally on the pyriform lobe (Fig 1j-k) were diminishing in amplitude.

The finding of positive potentials in the cephalic ventral end of the head of the caudate nucleus (Fig 2A) and along the external and internal capsules to the putamen, the globus pallidus and the entopeduncular nucleus (Fig 2B-D) may represent a pathway between the olfactory system and the striatum. At this time, however, we can make no more than passing comment concerning the possibility of such a connection. These responses were ob-

THE EFFECT OF CALCIUM ON THE NEUROMUSCULAR JUNCTION

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INTRODUCTION

It is WELL known that calcium plays an important role in processes connected with membrane excitability. The effect of a reduction of ionized calcium in the surrounding fluids has been studied in ganglia (5, 20, 6, 11) and the site of the resulting activity or depression was found to be in the ganglion cells and also in the preganglionic nerve endings. Excitability changes have been noted frequently in medullated nerves during calcium lack (13) and more recently oscillatory local potentials appearing after stimulation and also spontaneously have been shown in isolated giant nerve fibres (10, 2, 3). The effect of calcium lack resulting in neuro-muscular block was studied by Brown and Harvey (7) while alteration of drug action after reduction of calcium in the fluids surrounding the muscle was shown by Harvey (12).

The main purpose of the present experiments was to investigate the activity of nerve endings, of muscle fibres, and especially of the endplates during calcium lack. The transmitter action in that condition and during calcium excess could be studied conveniently in the single nerve-muscle fibre preparation, (16) while changes in the resting potential were observed on the whole isolated frog's sartorius. Many of the observations on isolated nerve-muscle preparations could be reproduced in cats and frogs by removal of the parathyroid glands (unpublished observations). The method of leading from isolated nerve-muscle fibre preparations at the interface and in paraffin has been described previously (16). The slowly developing and enduring potential changes in the sartorius muscles were recorded with the same technique as employed recently in similar investigations (19).

RESULTS

SECTION I

The spontaneous activity of isolated frog muscles in calcium-free Ringer solutions is well known. Such activity can be achieved also when calcium is reduced by addition of Na-oxalate or citrate (1).

1 Site of origin of discharges

In 17 out of 19 sartorius muscles spontaneous activity resulted after immersion into calcium-free Ringer solution. The muscles were first soaked in normal frog's Ringer and afterwards were suspended vertically in paraffin oil floating above a calcium-free Ringer solution. The muscles then were gradually lowered into the test solution below, the pelvic end first. At the

In a few instances positive potentials along the external and internal capsules and in the putamen, the globus pallidus and the entopeduncular nucleus were recorded. The significance of these responses are not known. They may represent a pathway from the olfactory system, possibly to the striatum.

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tential at the pelvic end was maintained and its time course usually developed as shown in Fig 1. In three muscles, however, the depolarization at the innervated zone started to diminish after 20–30 minutes of immersion and eventually this part of the muscle became positive relative to the rest of the normal preparation. The development of a negative injury potential at the 'normal' not immersed muscle part while the potential measurements were made, was not excluded at that time, such an event might explain the relative positivity of the immersed portion in the three muscles.

3 The effect on neuro-muscular transmission

Neuro-muscular transmission was greatly affected by reduction of the calcium content in the Ringer solution. Solutions of varying calcium content were obtained by mixing

normal Ringer (containing 0.03 per cent CaCl_2) with calcium-free solutions. The whole sartorius muscle was immersed into Ringer solutions of different calcium content and nerve-muscle transmission was tested every few minutes over periods up to 40 minutes. The nerve was stimulated by single or double shocks and electrical records were taken from a fixed position on the muscle. The muscle could also be stimulated directly at the nerve-free pelvic

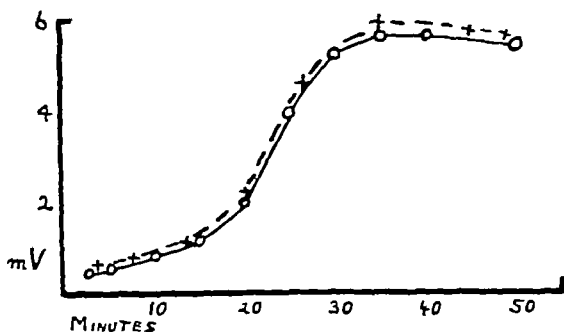


FIG 1 Time course of the negative potential change developing in parts of the sartorius immersed into a calcium-free Ringer solution. Crosses: potential change at the nerve-free pelvic part. Circles: at innervated zone of muscle.

end. The first result of calcium reduction was repetitive response following a single nerve volley. These were usually synchronous discharges followed by smaller responses mainly due to single fibre activity. This neuro-muscular hyperexcitability was succeeded, if the calcium was further reduced, by partial, and eventually by complete neuro-muscular block. During the block only the endplate-potential was recorded at the endplate regions after a nerve volley and even that disappeared gradually. At the same time the muscle spike set up by direct excitation of the pelvic end was not significantly altered in size and shape (Fig 2e). At this stage therefore, the main effect of the calcium reduction has been on the nerve-muscle transmission process and not on the ability of the muscle fibres to conduct impulses. The calcium content of Ringer solutions at which neuro-muscular hyperexcitability or depression occurs varies with different preparations. For instance immersion of the sartorius into Ringer solution containing $\frac{1}{4}$ of normal calcium concentration frequently produced neuro-muscular hyperexcitability over 10–15 minutes (cf. above). Subsequently the hyperexcitability disappeared and eventually partial neuro-muscular block resulted after 20–30 minutes.

same time two recording electrodes on the tibial end were connected through the amplifier to a loudspeaker or to a cathode-ray tube

When the pelvic 3-4 mm were immersed no activity resulted within 70 minutes (longest observation) Further lowering into the calcium-free solution is followed immediately or sometimes only after $\frac{1}{2}$ -1 minute by numerous discharges These continue for 20-25 minutes and then cease gradually After cessation of spontaneous activity discharges lasting for several seconds can be set up again if the muscle is stretched or stimulated directly In 3 cases some impulses were set up when only 4 mm of the pelvic part had been immersed into the calcium-free solution In each case it was later seen that some nerve branches reached very near to the pelvic end With the binocular microscope under suitable transmitted illumination and with the sartorius suspended in a shallow pool or floating on the saline solution, the extent of the innervation can easily be followed

The small size of the impulses and their asynchronous character suggested that most of the activity resulted from single muscle fibre impulses and was not due to nerve discharges This was proved by recording from the ventral roots during such activity, only occasional nerve impulses were back-fired Furthermore, even after paralytic curarization the spontaneous activity occurred (1) It is of interest that at times larger potentials, indicating the synchronous activity of several fibres, were seen This might have been due to chance, but it is also possible that synchronization occurs at adjacent endplates due to mutual influence of the partially depolarized regions (see below, Section I, 2)

Two additional experiments utilizing single nerve-muscle fibre preparations were performed On lowering these preparations into citrate Ringer solution, no discharges were observed until the nerve-muscle junction reached the solution Then, however, twitching occurred and persisted for 3-4 seconds This procedure could be repeated several times The effect of the citrate solution was unaltered after complete neuro-muscular block by curarine Thus it seems clear that during calcium lack in the surrounding fluid impulses are set up primarily at the endplate regions

2 Changes in resting potential

Slow changes in the muscle resting potential were measured during partial immersion into calcium-free solutions and solutions with their calcium content reduced to a varying extent The pelvic 3-4 mm or part of the innervated zone of the muscle could be immersed into the test solution while potential changes were measured relative to the normal muscle part in paraffin (19) The portion in calcium-free Ringer became gradually negative relative to the rest of the muscle (1) This applied equally when only the pelvic end was immersed and when part of the innervated zone was immersed Potentials up to 8 mV developed within about 20 minutes No significant differences of depolarization between muscle regions could be detected over this period In 9 experiments the change in the resting potential was followed for 70-100 minutes In all preparations the negative po-

gated muscle impulse due to summation of the two e p p's to above threshold value (Fig 2d), Further reduction of calcium diminished *pari passu* the e p p until it in turn disappeared. Even when the e p p was reduced to a fraction of its size as in Fig 2c, the muscle impulse (M), recorded at the endplate region, but set up some distance away from it by direct stimulation, was not appreciably different from normal (Fig 2b and e). This again is evidence that neuro-muscular transmission can be profoundly affected without appreciable alteration in the shape and size of the muscle spike.

The gradual e p p reduction in single nerve-muscle fibre preparations shows that the neuro-muscular block is due not to block of the nerve impulse before it reaches the nerve endings, but to the sub-threshold size of the endplate potential.

(u) The preparation was kept at the saline-paraffin interface with one recording electrode on the endplate (16). This method had the advantage that the calcium in the solution could be gradually reduced by addition of Na-citrate while at the same time photographic records could be taken at different stages of calcium reduction without altering the position of the electrode at the endplate. In these experiments, however, the actual calcium content of the solution surrounding the endplate was not known and it was not possible to record the muscle spikes set up by direct stimulation owing to the conditions of interface recording (16).

Figure 3b, c, and d illustrate some of the records taken at the interface during reduction of calcium by addition of Na-citrate. In Fig 3b a single nerve impulse evoked two muscle spikes. The second muscle impulse arose early in the relatively refractory period and is therefore small (17). The records immediately before (not illustrated) and after (Fig 3c) this exposure seemed quite normal. The initial part of the e p p preceding the spike and the late part after the spike does not appear to have been altered. No effect on the 'transmitter action' underlying the e p p component of the action potential (Fig 3c) is detectable at this stage (18). Further addition of Na-citrate reduced the late part of the negative potential after the spike. The e p p component outlasting the muscle impulse was

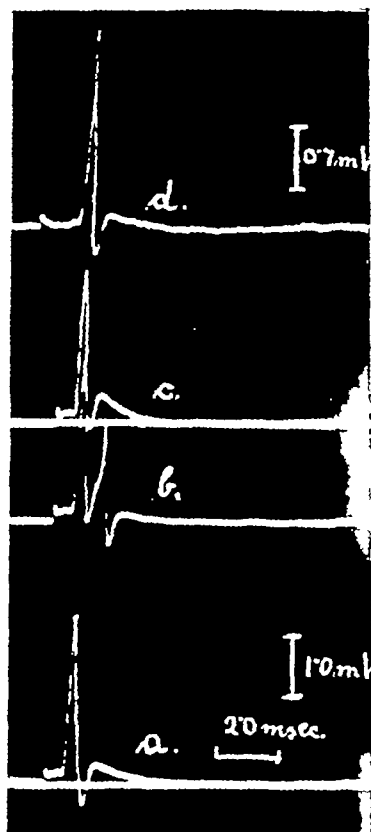


FIG 3 Potentials recorded from the endplate region in a nerve-muscle fibre preparation at the saline-paraffin interface during progressive reduction of calcium by Na-citrate. a, normal before Ca reduction, b, single nerve impulse sets up two muscle impulses, c, potential not appreciably different from normal, d, recorded shortly before transmission was blocked. Amplification is higher than in a, b and c. The negative potential following the spike is greatly reduced (see text).

All these different effects of the same solution, hyperexcitability or depression of transmission, are probably due to the slow diffusion of the test solution through the muscle substance which had been equilibrated in normal saline. While diffusion lasts there would be different calcium concentrations in the different layers of muscle fibres. The calcium reduction in the outer layers might be nearly complete while in other parts the calcium diminution is in its early stages. Thus all transitions of the effect of calcium reduction can be present in the same muscle simultaneously or in quick transition.

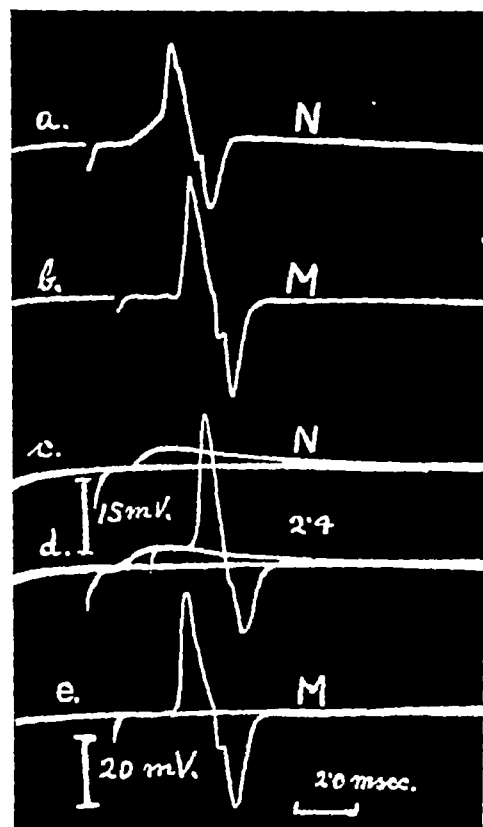


FIG 2 Action potentials recorded in paraffin oil from a single nerve-muscle fibre preparation with one electrode at the neuromuscular junction a, Potential set up by a nerve impulse (N), b, by direct stimulation of the muscle fibre some distance from the nerve-muscle junction (M). Further records taken after bathing the preparation for 3 minutes in $\frac{1}{4}$ of normal Ca content in Ringer c, single nerve impulse (N) sets up e p p only d, two nerve impulses at 2.4 msec interval set up propagating impulse (superimposed on single response) e, potential set up by direct stimulation (M) is not appreciably different from the normal at b. Amplification the same for a, b, and e (scale 20 mV), while at c and d the amplification is higher (scale 15 mV). Some of the records show the base line.

The effect of different calcium concentrations on the same junction was investigated by repeating the above experiments on four single nerve-muscle fibre preparations. In these preparations the effects of calcium lack disappear completely after 1–2 minutes washing in normal saline. The same preparation can be used to test different solutions with varying calcium contents in a short period. Two methods were found convenient for these experiments.

(1) The isolated fibre preparations were soaked in the solution for varying periods and then lifted into paraffin where the action potentials set up by nerve stimulation or direct muscle stimulation were recorded.

(2) The isolated fibre preparations were soaked in the solution for varying periods and then lifted into paraffin where the action potentials set up by nerve stimulation or direct muscle stimulation were recorded.

It was seen that reduction of the normal (0.03 per cent) calcium content in Ringer to $\frac{1}{4}$ or $\frac{1}{2}$ had a blocking effect on transmission. Under such conditions only an endplate potential (e p p) was set up at the neuromuscular junction (Fig 2c). Two nerve impulses at short intervals (2–5 msec) still set up a propa-

excitability of the muscle fibre at the junctional region, which now requires a longer lasting stimulation by the depolarizing action of the e p p (see accommodation and excitability changes in nerve [14]) Further increase in the calcium concentration eventually produced neuro-muscular block (Fig 4c) Summation of e p p 's still could restore transmission if the critical threshold value was attained (Fig 4d) During these stages of calcium excess the muscle spike itself did not seem to be significantly altered

A decrease in electrical excitability may be shown in curarized preparations where an increase in the calcium content (3-5 times) of the Ringer solution raises the critical potential at which the e p p fires off muscle impulses (9, 8, 15)

DISCUSSION

Investigation of the effect of calcium lack on the neuro-muscular junction has shown that during the first stages of calcium reduction the motor endplate region is strikingly and more selectively affected than the other parts of the nerve-muscle junction A differentiation of the nerve-muscle junction into nerve endings, endplate region and muscle fibre is made in view of different physiological responses from each of these structures

"Endplate region" in this sense might comprise more than the histological endplate How far the muscle immediately surrounding the endplate is affected for instance by a selective chemical action would be difficult to determine However, this "endplate region" differs strikingly from that part of the muscle fibre which does not possess endplates Thus a drug (acetylcholine, nicotine, caffeine) might depolarize the endplate region without appreciably affecting the rest of the muscle fibre and eventually a response could originate at the endplate (19)

There is no doubt that the excitability of medullated and non-medullated nerve fibres is increased after calcium reduction Spontaneous activity results when sufficient calcium has been removed from the surrounding fluid

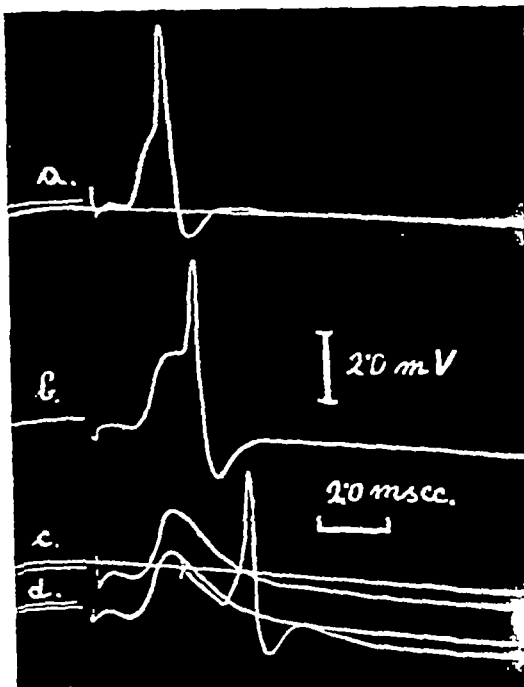


FIG 4 Action potentials recorded from the endplate region at the saline-paraffin interface a, before calcium was added to the saline b, c and d show progressive effect of Ca excess At c neuro-muscular block has occurred d, two nerve impulses at 2.4 msec interval set up a spike (superimposed on single response)

greatly diminished (Fig 3d) This action is similar to the subparalytic curarine-effect shortening the e p p duration Eventually block occurred due to reduction of the initial e p p which normally sets up the muscle impulse

Four isolated sartorius preparations were kept for up to 24 hours in calcium-free saline after the indirect excitability through the nerve had ceased Strong direct stimulation of the muscle still produced a weak contraction showing that even such extensive removal of calcium does not make the muscle entirely inexcitable

4 *Acetylcholine sensitivity during calcium lack*

Acetylcholine (ACh) sensitivity was tested by applying a small quantity of the drug (less than $3 \mu\text{l}$) to different regions of the innervated part of the sartorius (9) The impulses set up were mostly recorded by a loudspeaker system The whole or part of the sartorius was immersed into saline solutions of different calcium content The ACh concentration required to set up discharges was usually reduced from 100 to 1000 times after soaking the muscle in a Ringer solution of $\frac{1}{3}$ to $\frac{1}{5}$ of normal calcium content for several minutes This low threshold returned to normal in sometimes less than one minute after washing the preparation in normal Ringer

The lowering of the ACh threshold was frequently very transient, for instance after bathing the muscle for 3 minutes in $\frac{1}{4}$ normal calcium Ringer the threshold would be 100 times lower and after 5–6 minutes return to normal again This is probably analogous to the state of the isolated junction preparation which showed a transient hyperexcitability (repetitive response of Fig 3b), then returned to normal and was eventually blocked The locally applied ACh reaches only a few superficial endplates and these would rapidly undergo changes as does an isolated junction which is very quickly affected by the change in the surrounding fluid

It was also noted that discharges evoked by ACh during the period of lowered threshold lasted usually a few seconds longer than normally This would be expected because a nerve impulse at that stage also sets up many afterdischarges following the main muscle spike (Section I, 3)

SECTION II

Excess of calcium and neuro-muscular transmission

Calcium chloride was added to the Ringer solution while the neuro-muscular transmission was recorded at the endplate of a nerve-muscle fibre preparation placed in the saline-paraffin interface Figure 4 illustrates the progressive effect of calcium excess The first effect (Fig 4b) seemed to be a delayed origin of the spike set up by the initial e p p (the "normal" potential of Fig 4a was not recorded exclusively at the endplate for in that case no e p p "step" preceding the spike would be seen [16]) The only appreciable change in the e p p was its longer persistence before initiating the spike The simplest explanation would be to assume a decrease in the electric

parathyroids removed and showed signs of tetany (unpublished observations)

Calcium excess as seen in its effect on the isolated fibre preparations seems to block neuro-muscular transmission primarily by raising the threshold of excitation in the muscle fibre. The e p p has to persist longer to set up the propagated spike which itself is not altered in size and shape (Fig 4b). Also in this condition summation of e p p's can set up propagated responses, when one alone fails.

SUMMARY

The effect of calcium lack and excess on the neuro-muscular junction has been investigated in frog's sartorius and isolated nerve-muscle fibre preparations of the M. adductor longus.

- 1 Reduction of ionized calcium in the surrounding fluid affects the endplate region prior to the nerve endings and muscle fibres
 - (a) Spontaneous activity resulting after immersion into citrate or calcium-free saline solutions originates primarily at the endplates
 - (b) Reduction of calcium to $\frac{1}{3}$ – $\frac{1}{2}$ of normal first increases the excitability of the endplates as judged by repetitive response to a single nerve impulse. Also the sensitivity of the endplates to applied acetylcholine is 100–1000 times increased. Subsequently neuro-muscular block results while the nerve endings still conduct impulses and the muscle action potential set up by direct electric stimulation is not appreciably altered. It is suggested that the block is due either to (1) diminished production of the "transmitter," or (2) diminished electric excitability of the endplate region.
- 2 The part of the sartorius immersed into calcium-free or calcium-deficient saline becomes negative relative to the rest of the muscle.
- 3 Excess of ionized calcium gradually blocks nerve-muscle transmission presumably by lowering the electric excitability of the muscle fibre adjacent to the endplate.

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(13, 5, 20, 6) It is shown, however, in the present experiments that the endplate region in muscle responds before the nerve fibres discharge spontaneously

Further, Fessard (10) and Arvanitaki (2, 3) found that during calcium deficiency oscillatory potential changes were associated with discharges in giant nerve fibres of crabs. It was not possible to detect similar membrane changes at the endplate region following an excitation by indirect stimulation through the nerve or preceding spontaneous discharges. An attempt to detect the latter was made on isolated nerve-muscle fibres. The preparation held vertically suspended in paraffin oil was lowered into a citrate-Ringer solution while one electrode was kept at the end of the muscle fibre in paraffin, the other in the test solution, giving an effective lead at the saline-paraffin junction. When the endplate reached the citrate solution series of impulses were set up there and these could be observed on the oscillograph screen. No oscillatory potential changes were detected associated with these discharges. It is possible that the leading was not localized enough to detect changes which might be only a small fraction of the muscle spike size.

The findings regarding neuro-muscular block seem to be readily explained by the effect of calcium lack on the e p p size as seen in curarized muscles and normal single nerve-muscle fibre preparations. The gradual reduction of the e p p during calcium lack is similar to the curarine effect. Also a diminution of the "transmitter action" underlying the e p p (18) does probably take place. Such diminution may be correlated with findings on the superior cervical ganglion. There calcium lack causes a gradual block of transmission and at the same time spontaneous discharges are set up in the ganglion cells, a set of observations strikingly similar to those in the whole muscle. As the synaptic block develops a gradual diminution in the ACh output from the ganglion can be observed (11).

The responses to nerve stimulation and the size and time course of the e p p suggest that two phases occur during calcium lack. (i) Increased excitability of the endplate region and perhaps of the muscle. During this phase the e p p is not appreciably affected, but a single nerve impulse can set up repetitive responses. Also the ACh sensitivity is increased during that period (the same concentration of ACh causes a higher frequency of discharges from ganglia when calcium in the transfusion fluid is reduced [6]). (ii) block of transmission due to diminution of the e p p. During this state the diminished e p p's can sum and set up propagated responses if repetitive stimulation is applied.

The change in electrical properties known to occur during calcium reduction in nerve (21, 14) and probably in other tissues might also account for the present findings, but this is at present not amenable to direct investigation.

Many of the findings in isolated muscle, such as the specific excitability changes of the endplates were also found in cats and frogs which had their

EFFECTS OF DORSAL ROOT SECTION ON CHOLINE ESTERASE CONCENTRATION IN SPINAL CORD OF CATS

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INTRODUCTION

RECENT investigations suggest that formation of acetylcholine is intrinsically connected with the potential changes at the neuronal surface occurring during nerve activity (7, 16, 17, 18). Among the observations supporting this view is the finding of a localization of choline esterase at the neuronal surface and its presence there in concentrations high enough to remove acetylcholine at a rate comparable to that of the potential changes. If the activity of choline esterase is related to nervous function as intimately as suggested by this new concept, the degeneration of nerve fibers should lead to a decrease in this activity. That nervous degeneration is associated with diminished concentrations of the enzyme has been previously found in studies on the skeletal muscle of frogs and guinea-pigs (3, 4, 5, 12) and in the superior cervical sympathetic ganglion of cats (4). In the present paper, observations are reported in which the effect of nervous lesions upon the activity of choline esterase within the central nervous system has been investigated.

METHODS

The animals used in this study were mature cats weighing generally from 1.0 to 2.5 kg.

Operation. The animals were operated upon under full surgical anesthesia induced by intraperitoneal injection of sodium nembutal, aseptic conditions being rigorously maintained. Spinal laminectomy involving the 3rd to 7th lumbar vertebrae was performed and the dorsal roots sectioned at these five levels. In one group of animals, deafferentation was carried out on the right side only, while in a second group, the dorsal roots were severed on both sides. In every case, the root was sectioned extrathecally, central to the ganglion, and a piece of root about 2 mm in length was removed. Care was taken to avoid damage to the ventral roots or to blood vessels and no bleeding was observed during the actual deafferentation. Both the unilateral and bilateral operations were performed in one stage. The wound was always carefully closed in three planes and only those animals used in which the postoperative course was entirely uneventful. Postoperatively, the cats exhibited the usual signs associated with dorsal root section, including anesthesia of the skin areas, absence of the knee-jerks and other evidences of nervous impairment.

Sacrifice. Animals, both normal and operated, were sacrificed by exsanguination under deep etherization. The site of operation was re-exposed and section of the dorsal roots confirmed. The cord with its enveloping meninges and attached spinal roots was then carefully lifted from the vertebral canal and placed in a shallow dish of mammalian Ringer solution. Next, the cord was freed from the investing spinal theca and the dorsal roots of the 3rd through 7th lumbar segmental nerves traced centrally as they spread fan-wise to enter the dorso-lateral sulcus of the cord. That part of the cord into which the 3rd, 4th, 5th, 6th, and 7th lumbar spinal roots enter was cut away and all the dorsal and ventral rootlets were clipped off flush with the cord surface.

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* Now Lieutenant Commander, MC-V(6), USNR. The opinions or assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

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Preparation of the cord block for analysis This isolated block of cord, approximately 3 cm long was placed on the stage of a freezing microtome, the dorsal aspect uppermost, and frozen solid. Two vertical, transverse sections were then made through the block separating two smaller pieces of cord, designated as A and B, from the cephalic and caudal ends. These pieces, each approximately 1 cm long, were dropped immediately into a solution of 10 per cent neutral formalin. The middle part, at about the level of entry of the 6th lumbar roots, was used for chemical analysis. While still frozen on the microtome, it was divided into four wedges as follows. With a sharp wafer razor blade, a coronal slice was made through the length of the block at a level planned to be immediately dorsal to the central canal. This section divided the block into dorsal and ventral portions which were then separated into right and left halves by gently widening the dorsal median sulcus and ventral median fissure and cutting the central commissures with a fine pointed scissors. Thereby, four pieces were obtained, which will be referred to as the dorsal left, dorsal right, ventral left and ventral right quadrant wedges.

Chemical analysis The four separate quadrant wedges were then weighed and ground with quartz in Ringer solution and the activity of choline esterase determined by the Barcroft-Warburg manometric method as described previously (15). As in earlier reports, the activity of the enzyme is expressed as QChE, that is, the number of mg of acetylcholine split by 100 mg of fresh weight of tissue in 60 minutes.

Preparation of stained sections for determination of percentage of grey and white matter in the analyzed block Pieces A and B, after fixation in 10 per cent neutral formalin, were embedded in paraffin, sectioned at 6μ and the sections immediately above and below the analyzed block stained by Nissl's method, with hematoxylin and eosin or Cajal's method as used by Hoff (8). These two sections were projected on centimeter-ruled, uniform thickness paper at such magnification as to give an image about 18 cm across. The outline of the cord and central grey matter was accurately drawn and carefully cut out and the paper corresponding to the grey and white matter in the dorsal and ventral parts of the cord weighed separately. In this way, the ratio of white to grey matter in each quadrant wedge and in the cord as a whole was obtained, the value being taken as the average of the upper and lower sections. Care was exercised that the ratio of the weights of the dorsal and ventral quadrants of paper equalled that of the weights of the tissue actually used.

RESULTS

A Normal cats The QChE of the spinal cord at the level described above was determined in 10 unoperated cats. In Table 1 are summarized the values obtained for the four different quadrant wedges. The QChE of the grey matter is calculated from the observed QChE of the whole tissue. For this calculation, it is necessary to know the concentration of choline esterase in the white matter and the percentage of grey matter in the whole tissue. As found in a series of 8 determinations, the average QChE of white matter stripped in thin slices from the surface of the cat's spinal cord is 0.61. The determination of the ratio of grey to white matter is described above. One calculation may be given as an example. The QChE observed in the ventral left quadrant wedge of Cat No. 27 was 10.0. Since 40.5 per cent of this quad-

rant wedge is grey matter, $0.61 \times \frac{59.5}{100}$ or 0.36 must be subtracted from the

QChE of 10.0 to obtain the amount of enzyme in the grey matter alone. This is 9.64 for 40.5 mg or 23.8 for 100 mg of grey matter. In the tables are recorded the single QChE values observed for the whole tissue as well as the corrected average values for grey matter alone.

In the two dorsal quadrant wedges, which usually form between 40 and 50 per cent of the entire block, the concentration in the grey matter was, on

Table 1 Choline esterase in 6th lumbar segment of spinal cord, determined separately in four quadrant wedges

Normal cats

Cat no	Weight kg	Dorsal half in per cent of whole	Per cent of grey matter	Dorsal						Ventral					
				Left			Right			Left			Right		
				mg tissue	QChE		mg tissue	QChE		mg tissue	QChE		mg tissue	QChE	
					observ single	corr aver		observ single	corr aver		observ single	corr aver		observ single	corr aver
1		54.2	d 41 v 42	78.1	6.3 6.2	14.4	75.4	6.1 6.4	14.4	63.8	8.6 8.5	19.5	64.9	8.4 8.5	19.3
5		48.2	d 41 v 42	57.0	6.8 6.7	15.6	62.0	5.9 6.0	13.6	55.2	8.3	19.0	73.0	8.8 8.6	19.9
8		31.2	d 41 v 42	54.0	6.3 6.2	14.4	51.5	6.3 6.2	14.4	109.0	7.8 7.7	17.6	106.1	7.9 8.0	18.1
9	1.9	40.5	d 41 v 42	81.7	5.2	11.8	68.1	6.0 6.3	14.1	114.0	6.3 6.5	14.4	106.3	6.8 7.1	15.7
12		35.5	d 41 v 42	37.0	6.8 6.7	15.6	44.6	6.7 7.2	16.1	79.2	8.2	18.7	69.8	9.2 8.9	20.7
18		49.2	d 46.9 v 38.5	87.7	5.3 5.3	11.6	81.8	5.4 6.2	12.3	91.6	6.4	15.7	82.7	6.4 6.6	15.9
27	1.3	48.5	d 50.5 v 40.5	66.3	7.8 7.8	14.9	68.3	8.5 8.4	16.1	80.0	10.0	23.8	68.0	10.0 10.7	24.7
28	2.4	40.6	d 41.9 v 43.3	69.0	5.9 5.8	13.2	65.9	5.6 5.4	12.3	96.5	8.0	17.7	100.8	7.9 7.7	17.2
29	2.2	42.8	d 42.3 v 42.3	92.0	5.2 5.2	11.5	82.7	5.4 5.4	12.0	123.5	5.3 5.4	11.8	110.0	6.2 6.3	14.0
32	0.95	46.4	d 53.4 v 49.4	63.4	7.8	14.1	52.0	7.4	13.3	64.4	8.9	17.4	68.2	10.3	20.3
Average						13.7			13.9			17.6			18.6

Table 2 Choline esterase in 6th lumbar segment of spinal cord, determined separately in four quadrant wedges
Right unilateral deafferentation in cats

Cat no	Weight kg	Dorsal half in per cent of whole	Per cent of gray matter	Dorsal						Ventral					
				Left			Right			Left			Right		
				mg tissue	QChE		mg tissue	QChE		mg tissue	QChE		mg tissue	QChE	
					obsrv single	corr aver		obsrv single	corr aver		obsrv single	corr aver		obsrv single	corr aver
3	2.5	19.1	d 11 v 12	77.7	1.5 4.5	10.1	79.0	4.4 5.1	10.7	80.5	5.6	12.5	81.5	6.8 6.7	15.2
4	2.1	15.5	d 11 v 12	91.0	1.4 4.4	9.8	82.0	4.8 4.7	10.7	102.0	6.1 6.1	16.1	105.0	6.4 5.6	14.7
10	2.1	13.2	d 11 v 12	91.6	1.2 4.1	9.2	88.3	4.4 4.7	10.2	126.0	5.6 5.6	12.5	115.4	5.6	12.5
16	2.0	16.0	d 17.7 v 37.9	62.5	6.7	13.3	63.0	6.7	13.3	78.0	7.8	19.5	69.0	7.4	18.5
39	2.3	33.3	d 33.0 v 45.7	49.0	5.0	13.9	53.0	5.2	11.5	109.0	5.5	11.3	95.0	5.4	11.1
11		31.0	d 37.2 v 11.1	50.0	4.7	11.6	46.0	5.4	13.5	109.0	6.6	15.2	106.0	5.7	13.0
12	2.6	36.3	d 37.3 v 39.2	64.0	5.4	13.5	65.0	5.4	13.5	123.0	6.5	15.7	104.0	6.5	15.9
13	1.7	31.2	d 38.0 v 15.7	50.0	5.1	12.4	48.0	5.4	13.2	97.0	6.1	12.6	91.0	6.0	12.5
Average				11.7			12.1			14.4			14.2		

the average, 13.7 and 13.9 respectively, as compared with QChE of 17.6 and 18.6 in the two ventral wedges. The enzyme concentration is therefore about 30 per cent higher in the ventral than in the dorsal grey matter.

In individual animals the QChE values generally do not differ from the average by more than 20 to 25 per cent in either direction, variations well within the expected range. There is no obvious relation between the enzyme concentration and the total body weight. In view of the low concentration of choline esterase in white matter, the individual variations cannot be attributed to differences in QChE of white matter. It must be stated that the block was not always sliced at precisely the same horizontal level and this may possibly influence the QChE values since the enzyme is undoubtedly not evenly distributed in the grey matter. However, the differences found are much greater than could be explained in this way.

B Effects of deafferentation. In animals deafferented in the manner described above and sacrificed approximately two weeks later, the concentration of choline esterase in the spinal cord decreases about equally in all four quadrant wedges. The results of 8 experiments on cats with unilateral deafferentation and with bilateral dorsal root section are summarized in Tables 2 and 3. The QChE values for the grey matter are calculated in the same way as described for normal cats.

Although some values after unilateral or even after bilateral deafferentation overlap those obtained for normal animals, the average values definitely decrease following the section. After cutting the right dorsal roots, the QChE values show an average drop in the left dorsal quadrant wedge of 14.5 per cent and in the right dorsal wedge of 10.8 per cent. In the ventral wedges, the decrease in absolute values as well as in percentage is greater: 18.2 per cent in the left wedge and 23.7 per cent in the right.

The significance of these values is enhanced by the results of the bilateral deafferentation experiments in which the percentage decrease is roughly twice that found in the experiments with unilateral dorsal root section, namely, about 30 per cent in all four quadrant wedges. In Table 4 are listed the average QChE values of the grey matter in the four quadrant wedges of normal and operated cats. In the eight groups of quadrant wedges from operated animals which have been compared with the corresponding normal there is in every case a drop in the average choline esterase concentration. The likelihood of so consistent a change being due to chance alone is only 1 in 256 or 0.4 per cent. Comparing (by the method described by Fisher [p. 120]) all the QChE values obtained for the dorsal quadrant wedges after unilateral deafferentation with the figures for the corresponding wedges in all the bilateral experiments, a value of P of less than 0.02 is derived. (6) A similar comparison between the QChE values for the ventral quadrant wedges after the unilateral operation and those obtained after bilateral dorsal root section also yields a value of P between 0.01 and 0.02. The differences between the unilaterally and bilaterally deafferented cords may therefore be regarded as statistically significant while an even higher degree of

confidence may be placed in the differences between normal and operated animals

These decreases are the more striking when it is considered that in such a block of cord the dorsal roots contribute only a fraction of the nerve endings present and that there are a large number of synapses in the block which are the terminals of fibers ascending and descending from other levels

Table 4 Average values of QChE of grey matter in four quadrant wedges of 6th lumbar segment of spinal cord Values for normal cats are compared with those after right unilateral deafferentation and after bilateral deafferentation

Quadrant wedge		Controls (10 expts)	Right unilateral (8 expts)		Bilateral (9 expts)	
		QChE	QChE	per cent decrease	QChE	per cent decrease
Dorsal	Left	13 7	11 7	-14 5	9 7	-29 2
	Right	13 9	12 4	-10 8	10 4	-25 2
Ventral	Left	17 6	14 4	-18 2	12 2	-30 7
	Right	18 6	14 2	-23 7	12 3	-33 9

of the central nervous system Moreover, it should be remembered that the enzyme is concentrated not only at the nerve endings but also in the cell bodies That nearly one third of the enzyme present is localized within the afferent nerve endings is, therefore, remarkable

DISCUSSION

In 1937, Marnay and Nachmansohn demonstrated on the frog's sartorius muscle that at motor end plates there exists a concentration of choline esterase many thousand times as high as in the surrounding muscle fiber (10, 11, 13) Since that observation, the effect of nervous degeneration on choline esterase activity has been investigated Feng and Ting confirmed the results of Marnay and Nachmansohn and also examined the effect of section of the sciatic nerve on the activity of choline esterase in the toad's sartorius (5) They found a decrease in enzyme concentration of about one third in that part of the muscle containing the nerve endings In the gastrocnemius of guinea-pigs, Marnay and Nachmansohn observed an *increase* of enzyme concentration following degeneration of the motor nerve (12) This increase, however, is only apparent and must be attributed to the reduction in muscle fiber volume which occurs after section of the sciatic nerve (3, 4) Correcting for this by exact measurements of the change in volume with simultaneous determinations of enzyme activity, it was found that during the first 2 weeks, when the motor nerve endings disappear, the fall in enzyme concentration at the motor end plates is small and hardly measurable After 3 to 4 weeks,

Table 3 Choline esterase in 6th lumbar segment of spinal cord, determined separately in four quadrant wedges
Bilateral decerebration in cats

Cat no	Weight kg	Dorsal half per cent of whole	Per cent of grey matter	Dorsal						Ventral					
				Left			Right			Left			Right		
				mg tissue	QChE		mg tissue	QChE		mg tissue	QChE		mg tissue	QChE	
					observ single	corr aver		observ single	corr aver		observ single	corr aver		observ single	corr aver
13	2 0	37 4	d 45 5 v 47 0	54 1	4 9 4 9	10 0	44 8	5 3 5 3	10 9	78 6	6 2	12 5	87 5	5 9 5 8	11 8
14		42 3	d 44 9 v 39 3	56 5	3 0 3 0	6 0	58 3	3 6 3 5	7 2	81 3	4 4 4 4	10 2	75 6	4 7 4 4	10 7
15	2 5	40 4	d 47 3 v 49 9	55 5	5 0 5 1	10 0	59 6	4 0 4 1	7 9	88 1	5 9	11 2	82 1	4 3 4 2	7 7
16	3 8	50	d 44 1 v 36 9	78 5	4 4 4 5	9 3	73 9	4 8 4 7	10 1	74 8	5 8	14 5	76 1	5 8 5 7	14 5
20	2 0	39 3	d 45 5 v 46 6	61 9	6 7 6 6	13 9	65 8	6 3 6 5	13 5	102 8	6 2	12 5	95 1	7 3 6 9	14 5
23	2 4	37 1	d 34 8 v 39 0	56 3	5 3 5 3	14 0	43 8	6 0 5 7	15 7	83 4	5 4	12 9	86 4	5 1 5 2	12 2
25	1 7	42 5	d 51 1 v 41 7	44 9	4 6 4 3	8 1	43 2	4 8 4 8	8 8	55 2	5 2	11 6	63 8	5 4 5 3	12 0
26	1 6	46 6	d 50 4 v 42 0	61 3	4 5 4 4	8 2	57 5	6 1 5 9	11 3	65 2	6 5	14 6	67 6	7 7 7 2	16 9
30	2 2	41 2	d 45 6 v 46 3	66 1	4 0	8 0	60 8	4 2	8 4	88 1	5 0	10 1	92 9	5 3	10 7
Average				9 7			10 4			12 2			12 3		

zyme activity is obviously greater than could be expected from the concentrations found in the fibers. This suggests an increase of the enzyme concentration in the afferent fibers after they enter the spinal cord and must be attributed—as in the experiments with the superior cervical ganglion—to an increase of surface owing to the endarborization. It is difficult to believe that the mechanism of the action potential, *i.e.*, the propagation of nerve impulses differs fundamentally in afferent and efferent nerve fibers. The concentration of choline esterase is of the same order of magnitude in both dorsal and ventral roots indicating a similar rate of acetylcholine metabolism (15). As has been previously discussed, the fact that the presence of acetylcholine could not be demonstrated in dorsal roots appears inconclusive (9, 14). The present experiments offer additional evidence for assigning to acetylcholine the same role in the propagation of nerve impulses in both afferent and efferent fibers.

SUMMARY

1 The concentration of choline esterase in the grey matter of the 6th lumbar segment of the spinal cord in cats has been determined and the effects of unilateral and bilateral deafferentation on the enzyme activity have been studied.

2 In normal cats, the QChE values were, on the average, 13.7 and 13.9 in the left and right dorsal quadrant wedges respectively. In the left and right ventral quadrant wedges the values obtained were 17.6 and 18.6 respectively.

3 After unilateral deafferentation, a decrease of about 10 to 20 per cent was observed in all four quadrant wedges. After bilateral deafferentation, the percentage decrease was approximately twice as great, *i.e.*, about 30 per cent in all four quadrant wedges.

4 These results are compared with the effects of nervous degeneration on choline esterase activity in muscle and ganglia and the implications are discussed.

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when the end plates are already transformed into sole plates, the decrease amounts to 20 to 30 per cent, comparable to that found by Feng and Ting in toad's sartorius, where no complication due to volume changes in the muscle is encountered

In the superior cervical ganglion, Brucke reported a decrease of choline esterase activity after section of the preganglionic fibers (2) These observations, however, were not quantitative and were made under conditions open to criticism The substrate concentration was not high enough to saturate the enzyme and the rate of hydrolysis therefore was not optimal and not suitable for comparison Determinations made by Couteaux and Nachmansohn showed that the enzyme activity in the superior cervical ganglion of cats decreases about 60 per cent in 10 to 12 days, the time during which the nerve endings disappear (4) It then remains constant These experiments indicate that, as at motor end plates, a large fraction of the enzyme is localized outside the nerve endings

From the decrease of choline esterase activity within the ganglion—a decrease which is to be attributed to the degeneration of the preganglionic nerve fibers—it can be calculated that the enzyme concentration of the preganglionic fibers inside the ganglion is several times as high as in the same fibers before they enter the ganglion Owing to the extensive endarborization, the surface of the preganglionic fibers increases within the ganglion Therefore, the findings of Couteaux and Nachmansohn suggest that the enzyme is localized at the surface of the fibers Direct evidence for this assumption has been offered in experiments on the giant axon of the squid (1)

This localization of the enzyme at the neuronal surface may account for the relatively small reduction of the enzyme activity at the motor end plates after denervation of the gastrocnemius of guinea-pigs since the increase in surface area of these motor nerves at their endings is small compared with that of preganglionic fibers In the light of these previous results, the effect of section of the dorsal roots on the concentration of choline esterase within the central nervous system is particularly significant and indicates an extensive endarborization of the dorsal roots

The question may be raised whether the changes observed are not the results of an impairment of the blood supply of the cord produced by the operation rather than a direct effect of the nervous lesion This seems unlikely As stated above, great care was taken to avoid vascular damage and hemorrhage Also, it would not be easy to explain the symmetrical decrease of QChE in all four quadrant wedges were the lesion of vascular origin Moreover, histological control did not indicate any damage to the cell bodies in Nissl sections Finally, it is well known that localized ischemia of the cord is produced only with difficulty because of the rich anastomoses

A comparison of the decrease of QChE in the spinal cord block with the QChE of the dorsal roots themselves is of interest Only two determinations were made of the QChE of dorsal roots The values obtained were 0.27 and 0.31 However, even were the average values higher, the decrease of the en-

FUNCTIONAL ORGANIZATION OF FRONTAL POLE IN MONKEY AND CHIMPANZEE

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INTRODUCTION

DURING previously reported experiments upon the convexity of the hemisphere of monkeys and chimpanzees, electrodes had frequently been placed on portions of the frontal lobe lying anterior to area 8, and strychnine been applied in these same regions (4, 11, 6, 3, 1, 2, 9). In this manner much information had accumulated which served to simplify and orient the present experiments. In contrast to the structure and function of the posterior portion of the frontal lobe, where the cytoarchitectonic distinctions are clear and lesions produce definite results, this anterior portion presents extreme difficulties. Here in the monkey, Brodmann (8) could distinguish four areas but their boundaries remain ill defined and as yet no similar study on the chimpanzee has been published. Localized unilateral lesions of this region produce disturbances which are difficult to detect and, with the exception of the area orbitalis dysgranularis, electrical stimulation has yielded no clear-cut response. Systematic strychninization of the frontal poles with mapping of the cortical potentials derived therefrom has now been carried out on a series of monkeys and chimpanzees. The experiments in the monkey are scarcely worth reporting, for the method failed to define precisely or to disclose significant interrelations between the known cytoarchitectonic subdivisions. Therefore we wish to report in detail only the work on the chimpanzee.

METHOD

Two chimpanzees were used and these were fully anaesthetized with Dial Ciba* (0.35 cc per kg, one-half intraperitoneally and one-half intramuscularly). The animals were clamped securely to a board, the temporal muscle and zygoma were removed and the anterior two-thirds of the hemisphere exposed by wide craniotomy extending from the midline to the floor of the frontal and temporal fossae and from glabella to union. The dura was then incised and reflected widely, care being taken to preserve, insofar as was possible, the venous drainage as well as the pia mater. The whole preparation was then inverted so that the animal hung supine and the brain sagged away from the base of the skull. Area 8 was located by electrical stimulation and observation of eye movements (9). The stimulus was of moderate intensity and voltage but of long wave form and duration. Area 8 so defined served as the posterior boundary of the area to be investigated. 36 silver-silver chloride electrodes were then placed on the convexity of the frontal pole and 12 more on its orbital surface—all anterior to area 8. In each experiment a few square millimeters of filter paper moistened with a saturated solution of strychnine sulphate colored with toluidine blue were applied somewhere to the frontal pole, and the distribution of the induced disturbances was then mapped by recording the activity of all of the electrodes—6 at a time—by means of a Grass 6-channel oscillograph. The resultant sudden spike-like voltage, many times greater than the normal activity of the cortex, depends for its size upon the synchronous firing of many cells. It can be recorded wherever a sufficient number of these

* We wish to thank Ciba for placing the Dial at our disposal.

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less extent, the orbital surface. Figure 1B schematizes the axonal distribution disclosed by strychninization within the areas in question. The area having the widest axonal field corresponds approximately to the area lacking callosal connections, for both occupy a roughly circular area above the sulcus principalis and extend onto its inferior, or lateral, lip (3, 4). It fires into all areas on the convexity of the frontal pole. In sharpest contrast, the area at the tip of the frontal pole shows the most restricted firing on the convexity of the frontal pole, the disturbance extending only a millimeter or two beyond the area strychninized. Between these areas lies a narrow strip extending from the medial surface to the sulcus principalis. Strychninization in this strip causes disturbances throughout its length but not elsewhere. Between the area of maximal axonal field and area 8 lies a second narrow strip where strychninization causes disturbances propagated only inferiorly, or laterally, as far as the sulcus principalis. The remaining portion of the convexity lies below the sulcus principalis. It contains a large, posterior area extending forward from area 8 and upward from just below the orbital margin. Throughout this area the firing is antero-posterior and postero-anterior. Between this last area and the area at the tip of the frontal pole, and extending thence onto the orbital surface where it becomes adjacent to area 8, is another strip, characterized by firing not only into all parts of itself but also into the area of minimal firing at the tip of the frontal pole. The two remaining areas are narrow strips medial to that last described. The more lateral of these is the area orbitalis dysgranularis which fires all parts of itself but no other part of the frontal pole. Its connections to remote regions are described in an article on the long association tracts of the cortex (5). Finally, medial to this strip lies a narrow strip of cortex where strychninization causes firing of all parts of this strip but not of any other part of the cortex.

One more point must be mentioned—namely, that area 8, as manifest by eye movements and by suppression of electrical activity, extends from a point only a few millimeters dorsal to the sulcus calloso-marginalis, upward and thence laterally and then downward across the convexity of the hemisphere, and finally occipitally and medially on the orbital surface of the hemisphere, to disappear under the tip of the temporal lobe.

DISCUSSION

Neither the older histological studies of the cortex (8) nor those more recent stimulations which indicated that the eye field extended above the sulcus arcuatus (10), had suggested the extent of the eye field disclosed in these experiments. The type of stimulation used was designed to evoke responses with a minimum spread of current, and the eye field thus disclosed had a sharp margin. Its continuation on the orbital surface was extremely narrow. Nevertheless, one might have thought that this lower extension depended upon spread of current to some underlying tract, had it not been for the fact that strychnine caused a suppression of electrical activity

cells send their axones or collaterals. Thus this procedure discloses the axonal field of the cells in the area strychninized. From previous experiments upon the chimpanzee it was already known that strychninization of area 8 produced a suppression of electrical activity of the cortex. (1) This served as a check on the boundary of the frontal pole and was frequently, though unintentionally, confirmed in the present experiments. Also, from previous extensive experiments on the cortex of the convexity (4, 11, 6, 3, 1, 2, 9) and orbital aspect of the hemisphere (5), it was known that no strychninization of frontal regions posterior to area 8 caused strychnine spikes to appear in the frontal pole and that with few possible exceptions the same held true in the reverse direction. Finally, from the work on the commissural systems of the cortex (3, 4) it was already known that except for one area in the vicinity of what is presumably the sulcus rectus, or sulcus principalis, the convexity of the frontal pole had everywhere homotopic projections to the other hemisphere. This was confirmed again in the present experiments. The brain was photographed and a careful drawing made, on which the sites of the strychninizations and of the electrodes and the outlines of area 8 were plotted and the sulcus principalis identified. Then, on the basis of the axonal fields as defined by the propagation of strychnine spikes, areas having similar projection fields were plotted as the experiment progressed—thus facilitating the choice of position for the next strychninization.

RESULTS

On the basis of the axonal fields as defined above, it was at once possible to subdivide this entire region into eight areas, of which six appear on the convexity of the hemisphere, the lowest extending onto the orbital surface, and two others on the orbital surface alone. Figure 1A schematizes these fields on a diagram which is distorted to show both the convexity and, to a

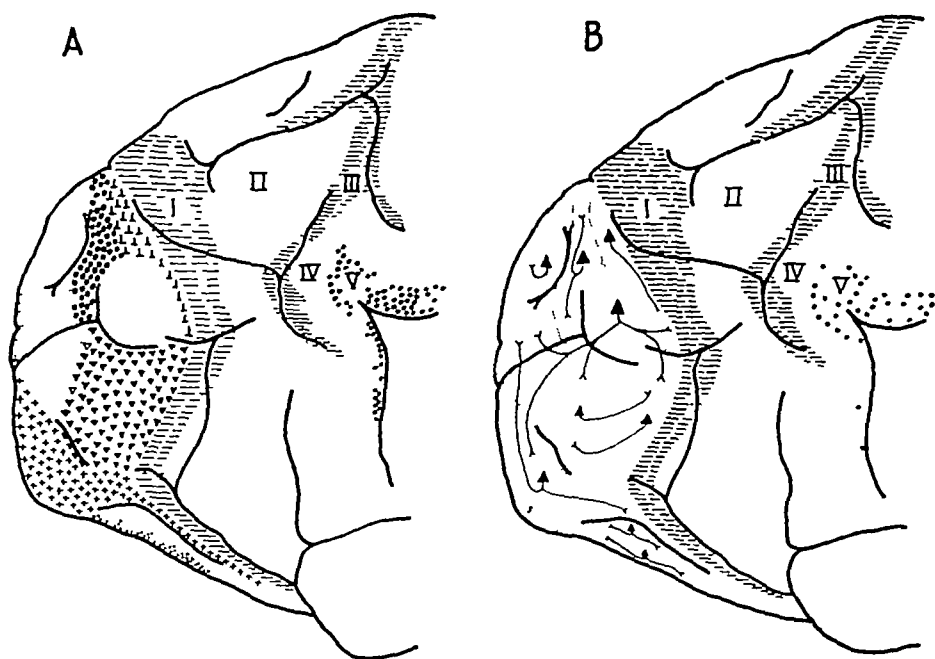


FIG 1 Functional organization of the frontal pole of the monkey and chimpanzee

THE DISTRIBUTION OF ACETYLCHOLINE IN BRAINS OF RATS OF DIFFERENT AGES

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ESTIMATIONS of the acetylcholine (ACh) content of different parts of the mammalian central nervous system have been made by a number of workers, employing a variety of animals (e g , 2, 9, 10, 21). A comparison of their results shows that, in general, the cerebellum has the least ACh of any part of the brain, while the region of the brainstem has the most. Only in a few cases have animals other than adults been used (7). The present paper offers an analysis of the distribution of ACh in four parts of the brain with reference to different developmental stages of the postnatal rat. It will be shown that the regional distribution of ACh is related to that of cholinesterase, also that the resistance of different parts of the mammalian nervous system to anoxia and hypoglycemia may be related to the quantitative distribution of ACh.

METHODS

Rats of the Sprague-Dawley albino strain were used. Rats referred to as "adult" weighed between 250–450 g, most being about 350 g. "Young" rats were 21–26 days old, and weighed 30–60 g. "Infant" rats were under 24 hours old, and weighed about 5 g each.

Animals were decapitated and the skull was removed dorsally, exposing cerebrum, cerebellum and medulla. The olfactory bulbs were removed and discarded. The cerebrum and underlying parts were separated from the cerebellum and medulla. The ventral portion was separated at the level of the lateral ventricles, this part, which included the mid-brain, thalamus, hypothalamus, corpora quadrigemina, and the optic chiasma, will be referred to as the "brainstem." That part of the cerebrum which remained, including the cortex, corona radiata, and corpus callosum, is referred to as pallium. The cerebellum was separated from the underlying pons, which, together with the medulla and whatever of the spinal cord was included, is called "medulla." Since decapitation was not always at the same level of the neck, the "medulla" was not included in the extracts of "whole brains" of different aged rats except where noted in Table IV.

The extraction procedure used was the cold-Ringer method described by Welsh (30), which extracts only the "free ACh," i e., that part of the total ACh readily extractible in water, in the presence of adequate amounts of eserine and without employing a protein denaturant.

Extracts were assayed on isolated hearts of the mollusc *Venus mercenaria*, because of its great sensitivity to small amounts of ACh. Details of the method of employing the Venus heart (based on findings of Prosser, 25) for assaying tissue extracts for ACh will be described in a separate publication.

A series consists of the ACh extracts of the four parts of a whole brain or group of brains (Tables I–III) or the extracts of whole brains of three or more different aged rats (Table IV). A series is to be found on a single horizontal line of a table. In assaying, the different extracts of a series were matched against each other to give relative values within the series, and also they were matched against known concentrations of ACh to give values in gamma per gram. Values given are in terms of equivalent weights of acetylcholine rather than of the salt. All of the extracts of a given series were assayed on the same heart, thus the relative values within a series are more reliable than those between different series.

exactly as it did elsewhere in area 8, and that strychnine is known to act only upon cell bodies

Strychninization of the frontal pole rostral to area 8 has revealed much greater differentiation and more complex interrelation of dissimilar areas than had been anticipated. The picture invites comparison with Brodmann's cytoarchitectonic map, not of the monkey but of man (7), for the areas disclosed here functionally in the chimpanzee are as numerous as the areas distinguished anatomically by him in man. Unfortunately, they are so different in shape and arrangement as to make it impossible to homologize them. Far greater correspondence exists between these areas in the chimpanzee and those distinguished by von Economo in man (12)—a correspondence which is enhanced by omission of those of his final subscripts which indicate a finer shade of cytoarchitectonic differentiation than can be confirmed with assurance.

The outstanding conclusion of the experiment is that with its relative increase in bulk the frontal pole of the chimpanzee, instead of resembling the ill differentiated frontal pole of the monkey, has come to resemble the highly differentiated and complex frontal pole of man.

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mated Values in gamma per gram of tissue were obtained for five brains, and the relative values found for the other six were in close agreement, as will be seen in Table III The results show that the parts in order of decreasing ACh content are brainstem, averaging 0.58 γ/g , medulla, 0.37 γ/g , pallium, 0.20 γ/g , and cerebellum, 0.10 γ/g , the differences being statistically significant Extracts of spinal cord and spinal nerves were made from five adult rats The results of the assays were, for spinal cord, 0.8, 0.8, 1.0, 1.0 and 1.5 γ ACh per gram tissue (av 1.0 γ/g) For spinal nerves (chiefly the

Table 3 The distribution of free ACh in the brains of adult rats

ACh, γ/g tissue				Relative values,* cerebellum = 1		
Brainstem	Medulla	Pallium	Cerebellum	Brainstem	Medulla	Pallium
0.8	0.50	0.10	0.08	10	6	1.25
0.6	0.45	0.2	0.1	6	4.5	2
0.7	0.4	0.28	0.1	7	4	2.8
0.3	0.28	0.15	0.05	6	5.5	3
0.5	0.25	0.25	0.15	3.3	1.7	1.7
—	—	—	—	7	5	4
—	—	—	—	4	3	2
—	—	—	—	9	5	2.5
—	—	—	—	5	3	2.5
—	—	—	—	5	6	2.5
—	—	—	—	2.5	3.5	2
Av 0.58	0.37	0.2	0.1	6	4	2

* Extracts of the different parts compared with one another but on the last six not matched with known concentrations of ACh

brachial and sacral plexuses) the results were 3.0, 3.0, 3.5, 3.5, and 4.0 γ ACh per gram of tissue (av 3.4 γ) Relative values for the parts of the nervous system of the adult rat are cerebellum, 1, pallium, 2, medulla, 4, brainstem, 6, spinal cord, 10, and spinal nerves, 34

To obtain an estimate of the quantitative relations of free to total ACh, whole brains of 8 adult rats were divided into right and left halves, one half being extracted for free ACh and the other half for total ACh The extraction procedures for total ACh were modifications of the method of Mann, Tennenbaum and Quastel (22) using $NHCl$, and the method of Chang and Gaddum (5) using trichloroacetic acid With both methods the tissues were ground with silica rather than minced Extraction with $NHCl$ gave values 2-3 times that with cold-Ringer, that with trichloroacetic acid gave 1.5-2 times that with cold-Ringer

D ACh content of whole brains of rats of different ages Whole brains of 6 series of different aged rats were extracted, and the level of free ACh in them was estimated The results, summarized in Table IV, show that infant rats (under 24 hours old) had an average of 0.1 γ free ACh/g brain, young rats (21-26 days old) an average of 0.2 γ , and adult rats an average of 0.4 γ From

RESULTS

A Distribution of ACh in brains of infant rats The brain parts of five groups of infant rats, under 24 hours old, were extracted and the level of free ACh was estimated. The parts of from 6–18 rats were pooled for a single series. The following average ACh values were obtained: medulla, 0.60 γ/g , brainstem, 0.28 γ/g , cerebellum, 0.26 γ/g , and pallium, 0.16 γ/g (see Table I). It will be seen below that the cerebellum has least ACh per gram

Table 1 The distribution of free ACh in the brains of infant rats

Number of rats used	Free ACh, γ/g tissue			
	Brainstem	Medulla	Pallium	Cerebellum
18	0.38	0.50	0.30	0.34
6	0.17	0.18	0.10	0.13
11	0.33	1.00	0.17	0.40
9	0.29	0.67	0.17	0.22
9	0.25	0.67	0.08	0.20
Average	0.28	0.60	0.16	0.26

of tissue in young and in adult rats, therefore, for comparison of relative values the cerebellum is taken as 1. The relative values of the other parts of the infant brain are: medulla, 2.3, brainstem, 1.1, and pallium, 0.6.

B Distribution of ACh in brains of young rats The brain parts of nine young rats, between the ages of 21 and 26 days, were extracted and assayed for free ACh. The results, summarized in Table II, show the following aver-

Table 2 The distribution of free ACh in the brains of young rats

Age in days	ACh, γ/g tissue			
	Brainstem	Medulla	Pallium	Cerebellum
21	0.6	0.8	0.08	0.04
26	0.8	1.2	0.3	0.15
25	1.2	0.8	0.2	0.19
26*	0.6	0.6	0.2	0.1
26*	0.6	0.6	0.2	0.08
23*	0.5	1.0	0.24	0.2
Av. 25 d	0.7	0.8	0.20	0.13

* Brainstems, cerebella and medullae of two rats were pooled and extracted together. The pallium of each animal was extracted and assayed separately as a control, in each case the assays gave identical values for the two pallia.

age ACh content: medulla, 0.8 γ/g , brainstem, 0.7 γ/g , pallium, 0.20 γ/g , and cerebellum, 0.13 γ/g . The relative values, if the cerebellum is taken as 1, are: medulla, 6.1, brainstem, 5.4, and pallium, 1.5.

C Distribution of ACh in brains of adult rats The brain parts of 11 adult rats were extracted and the level of free ACh in the different parts was esti-

MacIntosh (21) in his study of the distribution of ACh in the nervous system of the adult cat found the cerebellum lowest in total ACh ($0.18 \gamma/g$) and the autonomic nerves and ganglia highest ($15-40 \gamma/g$). Mixed spinal nerves gave values of $2.5-7 \gamma$ total ACh/g of tissue. Of the several parts of the brain the basal ganglia were highest in ACh ($7.0 \gamma/g$) while other parts gave values intermediate between cerebellum and basal ganglia. Barsoum (2) in his study of ACh in the brain of dogs obtained lowest values for the cerebellum and highest values for corpus striatum and optic thalamus. In the present study both of these regions were included in the "brainstem". Dikshit (9) likewise obtained highest values for basal ganglia of the cat and rabbit and lowest values for the cerebellum, with the gray matter of the cerebrum intermediate. These studies are sufficient to show that there is a close correlation between the distribution of free and total ACh in different parts of the brains of a variety of adult mammals. Similar studies have not been made on mammals of different ages, but Szepeswol and Caretti (28) in their study of ACh in the brain of the developing chick obtained results similar to those above on the rat. In the chick the medulla contains much ACh in the early embryo, which decreases with age, in the rat it is high in the infant, increases in the young and then falls quite a bit lower in the adult. In the chick the ACh in the diencephalon and mesencephalon increases from a medium amount in the early stages to a high level which is maintained in the adult, the ACh in the rat brainstem increases from infant to young, then there is a very slight decline in the adult. The cerebrum of the chick first has very little ACh, this increases and then falls off again, in the rat pallidum the amount increases, then falls off slightly. The chick cerebellum shows a medium amount of ACh in the early stages, this amount decreases in the older chick, in the cerebellum of the rat there is also a decrease in ACh with increased age. Thus there is a general agreement in the quantitative distribution of ACh in the nervous system of the developing chick and that of the growing rat.

Little attempt has been made to determine the possible functional significance of the different amounts of ACh in the several parts of the nervous system, nor has the quantitative distribution of ACh been shown to be related to any known property or function of the parts of the central nervous system. If the level of activity of the cortex is influenced by the level of ACh, as suggested by Welsh (30), it would be of interest to know whether the amount of ACh present in a unit mass of a portion of the brain is indicative of the normal relative activity of that part. It is possible that the observed differences in the ACh content of parts of the nervous system are due to variations in the amounts of active material (cell bodies, axons, dendrites) in relation to inactive (myelin, etc). No adequate data are available to settle this point but such differences would scarcely account for the spinal nerves of the rat having thirty times as much ACh as the cerebellum. It is of interest to attempt to relate the distribution of ACh to other known properties and functions of the parts of the mammalian central nervous system, and

Table I it will be seen that the free ACh in the parts of the brain of the infant rats are all greater than 0.1 γ/g , the value found for infant whole brains. To find a possible explanation for this discrepancy, the following experiments were carried out: several infant brains were extracted whole, the extraction taking 8 or 9 minutes from decapitation until the extract was in the centrifuge. The brains of several other infant rats were divided into four parts as in section a, and extracted separately, the procedure taking 25–30 minutes until the extracts were in the centrifuge. After centrifuging, the supernatant fluid from the four parts was pooled, this, when assayed against the extracts of "whole" brains, gave values 2–3 times as great. These experiments, in connection with the conclusion of Sykowski, Fazekas and Himwich (27) that there is a greater production of ACh by brain slices of the newborn than of the adult rat, offer a possible explanation for the differences between values for ACh in infant whole brains given in Table IV (0.1 γ/g) and those to be

Table 4 The free ACh content of whole brains of different aged rats

Free ACh, γ/g tissue			No infants used*
Adult	Young	Infant	
0.67**	0.33**	0.16**	(2)
0.4	0.2	0.12	(3)
0.30	0.12	0.06	(5)
0.4	0.2	0.1	(4)
0.44	0.20	0.10	(6)
0.18	0.12	0.08	(6)
Av. 0.4	0.2	0.1	

* 2 or more infant brains were pooled and extracted together

** In this series the medulla was included in the "whole brain". In the others the whole brains consisted of pallium, cerebellum and brainstem.

calculated from Table I (0.24 γ/g , calculated on the basis of percentage weights of the different parts).

DISCUSSION

The results of this study may be briefly summarized as follows: (i) different parts of the nervous system of the rat have widely differing levels of free ACh, (ii) the relative values of the different parts of the brain change with age, e.g. in newborn rats the pallium is lowest in ACh per unit mass and the medulla highest, while in adults the cerebellum is lowest and the brainstem highest, (iii) there is an increase in the free ACh per unit weight of the entire brain with age.

There are few determinations of free ACh in the central nervous system which may be compared with the above but several investigators have indicated that the amount of total ACh is in general one and one-half to four times the free ACh (7, 22, 30). The results obtained above on separate halves of brains of adult rats confirm this, the total ACh having been found to be 1.5–3 times the free ACh.

tioning of the brain. In the maintenance of adequate levels of ACh, either in the autonomic nervous system (3, 19) or the central nervous system (30) supplies of glucose and oxygen are necessary. In a perfused cervical ganglion of the cat if either of these is withheld the synthesis of ACh slowly declines. In the rat made anoxic by low atmospheric pressure, or hypoglycemic by insulin injection, the level of ACh in the cortex falls below normal. Since the cerebellum of the cat, which is lowest in ACh (21), is most susceptible to anoxia (26), and autonomic ganglia, which have the highest ACh content, are extremely resistant (1), it is of interest to examine more closely the order with which the various parts of the nervous system respond to oxygen lack. This has been done by numerous investigators who have observed the effects of anoxia on the histology of the cells of different parts of the nervous system, on the electrical activity of parts of the brain and on general behavior.

Dennis and Kabat (8) found that the Purkinje cells of the cerebellum of the dog are the first cells of the brain to be destroyed by the complete arrest of cephalic circulation. According to references cited in Cannon and Burket (4), concerning primarily the cat, the small pyramidal cells of the cerebrum survive anoxia for 8 minutes, the Purkinje cells of the cerebellum are destroyed after 13 minutes, the cells of the cerebral cortex are irreparably damaged if deprived of their blood supply for 10–15 minutes, while medullary centers survive 20–30 minutes and spinal centers 40–60 minutes. Sympathetic ganglia survive 60 minutes of anoxia, and the myenteric plexus, highly resistant to anoxia, survives for 2–3 hours. The superior cervical ganglion of the cat recovers, at least partially, after complete interruption of the circulation for 70 minutes (1).

After arrest of cephalic circulation the brain potentials of the cat disappear first in the cerebellar gray, next in the cerebral cortex, and last in the medulla, some portions of the brainstem are intermediate although others survive as long as the medulla (26). Gellhorn and Kessler (14) have shown a synergism of anoxia and hypoglycemia on cortical potentials of rats. In a series of investigations Gellhorn and coworkers (see 13, for references) have shown a depression of the somatic nervous system during anoxia while the excitability of the sympathetic system is increased. From observations on humans, by various workers, it has been shown that insulin hypoglycemia first affects the cerebellum and cortex and lastly the medulla (e.g., 17). Of the several parts of the nervous system it is apparent that the cerebellum and cortex are most susceptible to lack of oxygen and glucose and these parts are low in ACh and cholinesterase. Autonomic ganglia are very resistant to anoxia and are extremely high in ACh. Brainstem and medulla are intermediate in ACh content and in resistance to anoxia and hypoglycemia.

While a parallelism exists between ACh levels and resistance to anoxia in adult mammals there is an inverse relation in rats of different ages. Newborn rats are very much more resistant to anoxia than are adults (12) but the new born rat has only one-fourth as much ACh per gram of tissue in the brain

this will be done to the extent that available data, particularly on the rat, allow. There are data for the rat on the respiration of parts of the brain, on changes in respiration and glucose utilization with age, on distribution of cholinesterase in parts of the adult brain and in whole brains of rats of different ages, and on resistance of rats of different ages to anoxia and insulin hypoglycemia.

It has been shown (18, 29) that in the adult rat the respiration of the cerebral cortex or pallium is highest, the brainstem is next, then the cerebellum, and that of the medulla is lowest. Thus there is no direct correlation between the ACh levels and respiration of parts of the adult rat brain. Himwich, Baker and Fazekas (15), however, found that the excised cerebral tissue of rats 1 to 25 days old had a lower oxygen consumption than adult tissue. This was confirmed by Tyler and van Harreveld (29) who also demonstrated an increased glucose utilization with age. Thus the increases in respiration and glucose utilization with age are paralleled by an increase in the ACh level of the entire brain. The medulla of the newborn rat has the highest respiration of the several parts. This increases up to the fourth week and then falls off until in the adult rat the respiration of the medulla is the lowest. That of the brainstem is relatively low at birth, increases up to the fifth or sixth week and falls off slightly in the adult rat (29). These changes are paralleled by the changes in ACh found in the present study. Chesler and Himwich (6) have recently shown that the glycogen content of the older phyletic parts of the central nervous system (cerebellum, medulla, cord) of cats and dogs decreases with age, while that of the newer parts (cortex, caudate nucleus, thalamus) increases with age. Thus, except for the cortex, there is a parallelism between the changes in oxygen consumption, glycogen content, and ACh level of the older and newer parts of the brain with age.

A close correlation exists in the distribution of ACh and cholinesterase in different parts of the rat brain and in whole brains of rats of different ages. Nachmansohn (23, 24) found, in the adult rat, the lowest Q_{ChE}^* values (2.6–4.1) in the cerebellum and cortex and the highest (8.4–17.9) in parts of the brainstem. In the whole brain of a newborn rat he found a Q_{ChE} of 1.9, in a rat of 21 days this had risen to 7.2 and in a rat of 110 days to 10.5. The quantitative agreement is remarkably close as we have found the ACh of the adult brainstem to be six times that of the cerebellum and three times that of the pallium, while the ACh of whole brains of 21–26 day rats is twice that of newborn and the ACh of adults is four times that of newborn. A closer comparison of the several parts of the adult rat brain cannot be made as we divided the brain in a different manner from that used by Nachmansohn in his study of the distribution of cholinesterase. Nachmansohn (23, 24) has also determined the cholinesterase activity of parts of the brains of the rabbit, dog, ox and man. If the Q_{ChE} values for the gray and white matter of the pallium are averaged they are the lowest, and of the order of 1 to 2, while those of the caudate and lentiform nuclei are highest and of the order of 50.

A continual synthesis of ACh is probably necessary for the normal func-

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* QCM =mgm ACh split in 60 min by 100 mgm of fresh tissue

as does the adult. It does, however, have relatively more in the medulla. It is possible that the comparatively low level of ACh in the infant brain is due to the undeveloped state of the animal. The relative proportion of active nerve tissue (containing ACh) may be very much greater in the adult than in the newborn rat (11, 20). This may account for the relative increase in ACh per unit weight of brain with age. It has been suggested (16) that newborn animals can obtain anaerobic energy through glycolysis to a greater extent than adults. This, together with the fact that the infant possesses a rate of cerebral metabolism and oxygen utilization lower than that of the adult (18), provides an explanation for the greater resistance of infants to anoxia and may account for the inverse relation between ACh levels and resistance to anoxia in rats of different ages.

SUMMARY

Estimations of the free ACh of brain tissues of infant, young and adult rats were made, also of the spinal cord and spinal nerves of adults. In rats less than one day old the medulla was found to be highest in ACh ($0.6 \gamma/g$) and the pallidum lowest ($0.16 \gamma/g$). In adult rat brains the cerebellum was lowest ($0.1 \gamma/g$) and the brainstem highest ($0.58 \gamma/g$). In the adult rat the spinal cord was found to contain more ACh than any part of the brain and the spinal nerves more than the spinal cord. Taking the value of free ACh in the adult cerebellum as one, the other relative values are: pallidum = 2, medulla = 4, brainstem = 6, spinal cord = 10, spinal nerves = 34.

When whole brains of infant, young and adult rats were extracted and assayed the free ACh of the infant brain was $0.1 \gamma/g$, that of young rats was $0.2 \gamma/g$, while adults yielded $0.4 \gamma/g$.

An attempt has been made to relate the regional distribution of ACh in the mammalian nervous system to other known properties and functions of the parts of this system. Except for the pallidum, the changes in ACh level of the parts of the brain, with age, are fairly closely paralleled by changes in respiration, glucose utilization and glycogen storage. A close correlation has been shown between distribution of ACh and that of cholinesterase. It is pointed out that the order of increasing resistance of the parts of the nervous system to anoxia and hypoglycemia is essentially the same as the order of parts arranged to show increasing amounts of ACh per unit weight. That is, those parts which are least resistant to anoxia and hypoglycemia (cerebellum and cortex) are lowest in ACh, while those parts which are most resistant, at least to anoxia, are highest in ACh (spinal nerves, autonomic ganglia).

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FUNCTIONAL ORGANIZATION OF THE MEDIAL ASPECT OF THE PRIMATE CORTEX

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INTRODUCTION

THE MEDIAL surface of the cerebral cortex in various primates has been mapped cytoarchitectonically by Campbell (4), Brodmann (3), Vogt (13), and von Economo and Koskinas (7), and myeloarchitectonically by Mauss (9, 10). Marchi and retrograde degeneration studies (11, 14) of intercortical or thalamocortical connections have not been done extensively on this portion of the cerebrum.

The purpose of the present study is to determine the physiologically distinguishable areas on the medial surface of the cerebral cortex by their inter- and intra-hemispherical cortico-cortical connections. A study of the medial surface of the occipital region has been presented elsewhere (2).

METHOD

The functional organization of the medial aspect of the cerebral hemisphere was investigated by the method of physiological neuronography in 3 macaques (*Macaca mulatta*) and 4 chimpanzees (*Pan satyrus*), all under Dial† anaesthesia. In order to expose the medial surface, the cerebral hemisphere was widely exposed, the veins of the pia mater emptying into the superior longitudinal sinus were coagulated and severed. After these preliminary operations it was possible to expose the medial side by putting the animal, which was firmly tied to a board, on its side, and allowing the brain to sag out. In one macaque and one chimpanzee both hemispheres were exposed in order to explore the commissural connections. In two other macaques the corpus callosum was transected and the anterior tubercle of the thalamus exposed in order to investigate the corticothalamic connections. The technique of physiological neuronography has been described so frequently in this journal (1, 6) that it seems unnecessary to repeat the details.

RESULTS

The following areas could be identified in both the macaque and chimpanzee on or adjacent to the cingular gyrus.

1. Most of the anterior part of the gyrus cinguli consists of a suppressor area. Electrical stimulation of this area provokes a relaxation of existing muscular tension and abruptly stops a motor after-discharge in a most dramatic manner. On local strychninization, this area fires itself restrictedly, i.e., spikes (rapid high fluctuations of voltage) occur in the neighborhood of the strychninization but not throughout the whole extent of the area which

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‡ We wish to thank Ciba for placing the Dial at our disposal

minization of this area evokes spikes in the anterior tubercle of the thalamus in the macaque

3 On the posterior and inferior part of the gyrus cinguli, hugging the splenium of the corpus callosum, there is a small area which fires only locally in the cortex but projects to the anterior tubercle of the thalamus in the macaque

4 There is a narrow "belt" on either dorsal or ventral lip of the sulcus cinguli. To this belt spikes are propagated from all suppressor areas thus far identified—*i.e.*, from the anterior limbic area just described under 1, as well as from areas 8s, 4s, 2s, and 19s. There is some evidence that these different areas fire different parts of this belt. On the frontal lobe, this strip broadens to become a fairly large area extending just over the dorsal margin of the hemisphere. In the chimpanzee its ventral border is formed by a rather constant frontal branch of the cingular sulcus, while its dorso-occipital border runs at a distance of about 2 cm from this branch. In the parietal lobe the "cingular belt" broadens again, occupying the anterior part of the precuneus. Spikes are projected from any to every part of this belt. The anterior part of the belt adjacent to the cingular suppressor area has commissural connections, none was found for the posterior part.

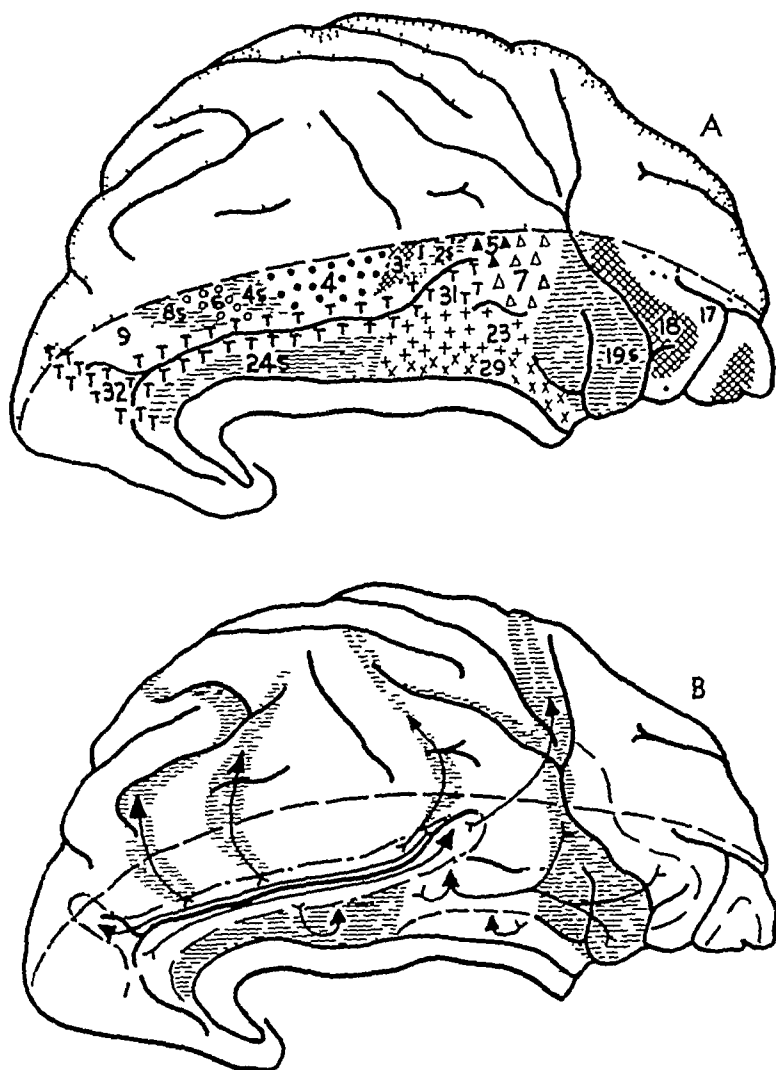
On the dorsal part of the medial aspect, between the cingular sulcus and the dorsal margin of the hemisphere, areas 8s, 6, 4s, 4, 3, 1, 2s, 5 and 7 were identified.

Certain areas, identifiable on the medial surface of the frontal lobe of the chimpanzee but not of the macaque, will be mentioned in the discussion.

DISCUSSION

It is not easy to homologize all the areas just defined by their cortico-cortical connections with those previously defined by their cytoarchitectural structure. The suppressor area in the rostral part of the gyrus cinguli, defined under 1, is readily identified with Brodmann's area 24, Rose's mesocortex (12) and v. Economo and Koskinas' area LA. The postero-dorsal cingular area described under 2 is identifiable as Brodmann's area 23, or v. Economo and Koskinas' LB. Similarly, the area of local firing close to the splenium of the corpus callosum, defined under 3, is undoubtedly one of the retrosplenial formations recognizable by their cytoarchitecture. It is impossible to say which of the three or four small areas described in that part of the primate brain is actually responsible for the phenomena observed, as each is too small to allow accurate topical strychninization. In lower forms, *e.g.* in the bat, where this part of the brain is much better developed, it is area 29 which forms the major portion of this region. In the primate brain, therefore, we shall allude to this area of restricted firing as area 29. It is difficult to identify the "cingular belt" on cytoarchitectural maps. All students of cytoarchitecture agree that its anterior part is "agranular" while its posterior part shows a well developed internal granular layer. The anterior part of the belt may be area 32, the posterior part area 31 as described

yields suppression. Firing is observed from one suppressor area to only the homologous small focus on the opposite hemisphere. From all parts of this area spikes are projected to the cingular belt (see 4). In the macaque, none is projected to the anterior tubercle of the thalamus.



Hemisphere of *Macaca mulatta*, showing (A) physiologically distinguishable areas and (B) their interareal connections

2. On the posterior and superior part of the gyrus cinguli there is an area which upon local strychninization fires itself widely and from which spikes are propagated into the pre-occipital and parastriate areas. These are roughly synonymous with Brodmann's 19 and 18 respectively. (2) Strych-

gous with areas 32 and 31, which receives connections from all known suppressor areas of the cortex (24s, 8s, 4s, 2s and 19s) but does not project to them. Area 32 has commissural connections, but none has been demonstrated for area 31.

Areas on the medial surface of the frontal lobe of the chimpanzee, which may correspond to Brodmann's areas 10, 11 and 12, have been identified and their firing characteristics determined. These were not identified in the macaque.

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by Brodmann in the human brain. The ventral boundary of the "belt" is more dorsal than that of Brodmann's area 31. Brodmann's map of the macaque bears little resemblance to our findings. In the brain of the cercopithecus, Mauss (9) described myeloarchitecturally a belt-like area 31 which occupies a position very similar to that of the cingular belt described here. That area 32 shows commissural connections, while area 31 does not, may be correlated with the difference in cytoarchitectural structure. Yet the two areas have the same ipsilateral cortical connection. All known suppressor areas fire into them, and together they may provide an important parieto-frontal and fronto-parietal pathway.

It is clear from our findings that the gyrus cinguli may be divided into at least three parts. Area 24 forms the anterior cingular region, for which in primates no thalamic radiations have been demonstrated. In rodents it receives them from the anteromedial thalamic nucleus (5, 8). In the macaque areas 29 and 23 form a posterior cingular sector from which there is a projection to the anterior thalamic nucleus. Areas 31 and 32 form a superior cingular region, it is impossible at present to define it in terms of cortico-thalamic connections. Although the "cingular belt" receives impulses from all suppressor areas, obviously areas 32 and 31 are not responsible for suppression, for strychninization of these areas does not lead to suppression of spontaneous cortical electrical activity, nor does electrical stimulation of them lead to suppression of motor response.

In the chimpanzee, at the tip of the medial surface of the frontal lobe we have distinguished, ventral and anterior to area 32, an area which fires area 32 but is not fired by it, this rostral area may correspond to Brodmann's area 10 in man. The suppressor area (24) extends around anterior and ventral to the genu of the corpus callosum. Just in front of the lamina terminalis is an area which we have not been able to expose well enough to determine its firing characteristics, here Brodmann locates his area 25 in man. Along the ventral margin of the medial surface of the human frontal lobe lies Brodmann's area 11, this region, in the chimpanzee, fires only locally but is fired by areas 21 and 22 of the temporal lobe. This leaves a central region, which has only been found to fire locally and corresponds to Brodmann's area 12 in man. Of all these areas, only area 10 fires across to the opposite hemisphere. We have not been able to distinguish these areas in the macaque.

CONCLUSIONS

By physiological neuronography the following areas on or near the gyrus cinguli of the macaque and chimpanzee were identified. Area 24 in the anterior part of the gyrus is a suppressor area. Area 23, in the posterior and superior part, sends impulses to the preoccipital and parastriate areas and projects to the anterior nucleus of the thalamus in the macaque. Area 29, close to the splenium of the corpus callosum, was not found to have other cortical connections, but also projects to the anterior nucleus of the thalamus in the macaque. Along the sulcus cinguli there is a "cingular belt," homolo-

OPTIC NERVE REGENERATION WITH RETURN OF VISION IN ANURANS

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THE RECOVERY of normal vision after regeneration of the optic nerve demonstrated in adult urodele amphibians (8, 9) requires that the ingrowing optic fibers reestablish in the brain centers discriminative functional associations which are differentially suited to the diverse retinal points from which the optic fibers arise. If the relationships between retinal field and brain centers formed in regeneration were disorderly or undifferentiated, normal vision involving discrete perception of small objects and their accurate localization in space would be impossible.

Conceivably, the central reflex relations as reestablished after regeneration might not be suitably arranged at first, but become properly adjusted only later through experience by a process of trial and error or other means of functional adaptation. Or the adequacy of the functional effect might somehow operate during the process of regeneration to regulate the formation of appropriate central connections. Both these theoretical possibilities, however, have been ruled out in the case of the newt, *Triturus viridescens* (8), in which it has been found that the restoration of normal vision after optic nerve regeneration is quite independent of functional adaptation.

When severance of the optic nerve in this animal is combined with 180 degree rotation of the eyeball on its optic axis, visual perception after recovery is systematically reversed about the optic axis corresponding to the rotated position of the retinal field. Reversed optokinetic reactions, erroneous spatial localization of small objects, and other clear indices of reversed vision are displayed consistently and without later adjustment, just as in animals in which the eyeball has been rotated with the optic nerve left intact (7). Thus in *Triturus* reestablishment of reflex relations in the visual centers is apparently predetermined in an orderly manner by growth factors regardless of the suitability of the functional effect for the animal.

Exactly how linkages between retina and brain centers are systematically restored by growth processes remains to be demonstrated. The work on *Triturus* suggested certain possible interpretations, however, and because of their important bearing on problems of broader significance concerning the developmental differentiation and integrative action of the nervous system it became strongly desirable to make sure that these results were not due merely to peculiarities of this one species.

The present paper deals accordingly with an extension of the previous experiments on the newt to several species of the distantly related and, so far as the visual system is concerned, more highly developed anuran amphi-

EXPERIMENTS ON TADPOLES

The regenerative capacities of anurans being less great than those of urodeles, it was not certain at the start of the experiments to what extent visual function might be recovered, if at all, after section of the optic nerve. The experiments were therefore begun on tadpole stages in which chances of recovery would be greater than in adults.

Operations In ten control cases (5 unilateral and 5 bilateral) in which the eye was left in normal position, the optic nerve with its sheaths was broken with jewelers' forceps. The stumps of the broken nerve floated rather freely in the fluid of the orbit and although some attempt was made to bring the ends close together before coagulation occurred, the broken ends in the majority of cases remained separated by a distance greater than the diameter of the nerve. Seventeen experimental cases were also prepared (7 unilateral and 10 bilateral) in which all ocular muscles were severed and the eyeball was rotated on its optic axis through 180 degrees. Several days later the nerve of the rotated eye was sectioned as in the control cases. A dorsal approach through a longitudinal incision over the eye was used both in sectioning the nerve and in rotating the eyeball. The unoperated eye of the unilateral cases in both the control and experimental groups was excised.

Recovery During the first week after operation no optokinetic reactions could be elicited in any of the animals. The first definite signs of recovery appeared on the average 13 days after nerve section but the recovery intervals varied in different cases from 11 to 23 days. One unilateral control case never recovered vision and two bilateral experimental cases recovered vision on only one side. Histological examination revealed that in these three exceptional instances the regenerating optic nerve had not succeeded in reaching the chiasma. Surgical readjustment of the eyeball, which had slipped out of its intended position, was necessary in two experimental cases.

Tests of vision The tadpoles in water in a fingerbowl were placed on a stationary platform inside a revolving upright cylindrical drum 31 cm. in diameter with opaque vertical black and white stripes on the inner wall. The stripes varied randomly in width from 2 to 7 cm. The optokinetic reaction was found to be readily and consistently elicited with this apparatus. After recovery was well established the animals were tested regularly over a five-day period and at weekly intervals thereafter. The 9 control cases and 16 experimental cases in which vision was successfully recovered displayed good optokinetic responses conforming with the following descriptions.

Results with unrotated eye Reactions of the bilateral control cases after recovery were quite like those of normal animals. The optokinetic response, consisting of an alternate beating of the tail with the strong beats in one direction, turned the head and body in that direction in which the visual field was revolving. Sometimes the animals turned in small circles in a stationary position and at other times they swam in larger arcs and circles, always in the direction of drum rotation.

The responses of the unilateral control cases were like those of normal animals from which one eye had been excised. Reactions when drum rotation was toward the blind side were normal but those with drum rotation toward the seeing side consisted usually of only a slow sustained flexion of the tail not strong or sudden enough to cause any movement of the head and body.

bians, the frogs and toads Included also are the results of some attempts at further analysis of the problem, namely, the effects on vision of localized lesions placed in the optic lobes of the brain before and after optic nerve regeneration

PROCEDURE AND MATERIALS

Plan of the experiments The general procedure was similar to that used previously in the case of *Triturus* Severance of the optic nerve was combined with 180 degree rotation of the eyeball on its optic axis With eye rotation the character of visual perception after regeneration of nerve connections between retina and brain centers might be (i) normal, (ii) reversed about the optic axis corresponding to the reversal of the retinal field, or (iii) randomly blurred If the recovered vision turned out to be normal in quality, despite the reversed position of the retinal field, it would be strong indication that function is of primary importance in regulating establishment of the central connections Recovery of reversed vision, on the other hand, would show that the original retino-central relations are systematically restored in a predetermined manner regardless of functional effect If visual perception on recovery should prove to be neither normal nor systematically reversed but instead a blurred confusion, it would indicate that redistribution and termination of the regenerating fibers is disorderly and nonselective, as in peripheral nerve regeneration (14)

The optic nerve was purposely pulled and teased apart in a rough manner in all cases rather than cut cleanly in order to prevent any neat approximation of the ends of individual fibers All operations were performed under ether anesthesia with aid of a dissecting microscope with magnification of 21 times After operation the tadpoles were kept at room temperature in 7-liter aquaria, and the frogs and toads in moist terraria of the same size

Animals Six species from three different families of anurans (*Bufo terrestris*, *Hyla cinerea*, *H. crucifer*, *H. squirella*, *Rana clamitans*, and *R. pipiens*) were used in the experiments All the animals were gathered in northeastern Florida The specimens of *R. clamitans* were undergoing metamorphosis when brought into the laboratory and were operated upon shortly after All the other adult animals had attained full size at the time they were gathered during their respective breeding seasons The tadpoles were operated on in mid-larval stages Although not identified with certainty, they were very probably all *H. crucifer* In general the differences in the results obtained on different species were not of sufficient importance for the essential problems concerned to warrant burdening the reader throughout with the species name of each animal mentioned Hence only the species and numbers included under each main treatment will be indicated and the species name of individual cases will be stated only where the results gave reason to believe that there might exist some significant species difference

Criteria of vision The ability to localize small objects in space was the principal index of visual perception used in testing recovery in the adult animals The accuracy with which frogs, particularly the tree frogs, gauge distance and direction in leaping for prey is quite remarkable (3, 5) *H. cinerea* was frequently observed in the course of this study to capture with a single leap houseflies walking at a distance of 35 cm, a comparatively easy feat in view of some reports This is mentioned only to furnish some indication of the efficiency of the anuran visual system No attempt was made in the present experiments to determine the limits of such performances The experimental tests were generally made at distances between 5 and 15 cm Discrete localization of small objects in different sectors of the visual field furnishes of course an excellent index of the functional properties or "local signs" of different retinal areas

In the tadpoles the optokinetic response to rotation of the visual field served as the chief criterion of visual function Although perhaps not indicative of so great a degree of specialization in retino-central associations as is the spatial localization of small objects, the optokinetic reaction is nevertheless dependent on a certain systematic differentiation of central reflex relations Therefore, its recovery after optic nerve regeneration, like the recovery of spatial localization, requires an orderly reestablishment of specific functional linkages between periphery and central nervous system which was the main concern of the present experiments

¹ For aid in identification of the animals acknowledgment is due Charles M. Bogert of The American Museum of Natural History

Recovery Functional regeneration of the optic nerve, though not so consistently successful as in the adult urodele, did occur readily in the great majority of these adult anurans. The first indications of recovery of vision began to appear on the average about 25 days after nerve section. The recovery period varied in different cases from 21 to 33 days. In two of the experimental cases the rotated eye became necrotic and was sloughed off. Two other experimental cases failed to recover vision in the rotated eye and two of the control cases recovered vision on only one side. The eye in these latter four instances retained a healthy external appearance, but microscopic examination revealed that regeneration of the optic nerve had been defective. Except for a very fine strand of fibers in one case the regenerating axons had frayed out along aberrant courses and had failed to reach the chiasma. In a fifth experimental case some signs of response to visual stimuli reappeared but the responses were too weak, infrequent, and inconsistent to permit any conclusions. A substantial strand of fibers about $\frac{1}{2}$ the size of the distal nerve stump was found in this case connecting with the chiasma but many small bundles of fibers had misregenerated along nearby structures of the orbit. The remaining 14 experimental cases which showed successful return of vision included 2 *B. terrestris*, 3 *H. cinerea*, 2 *H. crucifer*, 2 *H. squirella*, 3 *R. clamitans*, and 2 *R. pipiens*. The eight control cases included 1 *B. terrestris*, 1 *H. cinerea*, 2 *H. crucifer*, 2 *R. clamitans*, and 2 *R. pipiens*.

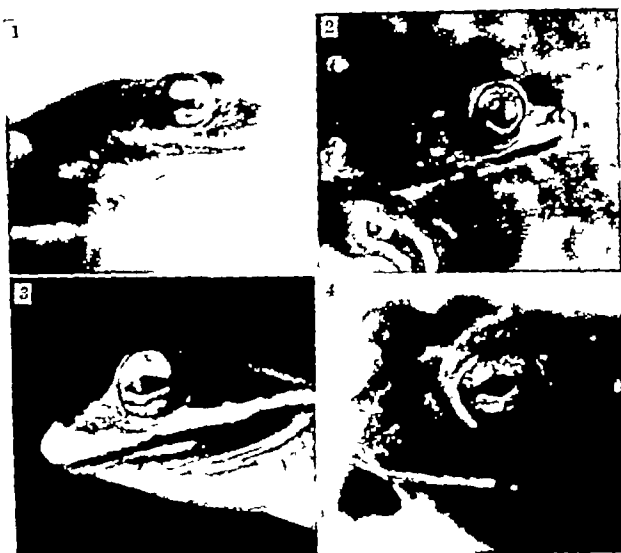


FIG 1 Appearance of eye after 180 degree rotation in four species of anurans 1 *H. squirella* 2 *R. clamitans* 3 *H. cinerea* 4 *B. terrestris*

ing axons had frayed out along aberrant courses and had failed to reach the chiasma. In a fifth experimental case some signs of response to visual stimuli reappeared but the responses were too weak, infrequent, and inconsistent to permit any conclusions. A substantial strand of fibers about $\frac{1}{2}$ the size of the distal nerve stump was found in this case connecting with the chiasma but many small bundles of fibers had misregenerated along nearby structures of the orbit. The remaining 14 experimental cases which showed successful return of vision included 2 *B. terrestris*, 3 *H. cinerea*, 2 *H. crucifer*, 2 *H. squirella*, 3 *R. clamitans*, and 2 *R. pipiens*. The eight control cases included 1 *B. terrestris*, 1 *H. cinerea*, 2 *H. crucifer*, 2 *R. clamitans*, and 2 *R. pipiens*.

Tests of vision The ability of these 22 animals to localize objects in space was tested regularly over a ten-day period beginning about one week after the first signs of recovery of function. A minimum of 8 trials per day was recorded for each animal. A housefly impaled on the end of a thin wire set endwise in a glass rod handle served as the lure. This lure was presented in different sectors of the visual field in random order. It was held with a slight oscillatory motion because the animals apparently strike almost exclusively at moving objects. Care was taken in testing visual localization to eliminate sensory cues other than visual and to avoid misleading reflections of the test object from the glass walls of the containers.

Results with unrotated eye After recovery the control cases with eyes in

Results with rotated eye The reactions of the tadpoles with rotated eyes after recovery were essentially like those of normal and control animals except that the direction of the responses was reversed. Instead of moving the head in the same direction in which the visual field moved, the animals turned in the opposite direction. The unilateral cases responded normally toward the blind side and made only a slow, sustained tail flexion toward the seeing side just as did the unilateral control animals, but the direction of drum rotation which elicited these responses was the opposite from that which was effective in evoking the same responses in the control group.

Reversed vision was also indicated in these animals by spontaneous cirrus locomotion. The bilateral cases swam in circles either clockwise or counterclockwise depending on how they happened to start. The unilateral cases circled with the blind side toward the center of the arc or circle. This tendency to swim in circles was comparable to that shown by *Triturus* after rotation of the eye (7). Both the reversed optokinetic responses and the spontaneous circling movements had been displayed in similar form by the bilateral cases during the few days immediately following eye rotation prior to optic nerve section. The results show that in these anuran larvae just as in adult *Triturus* the central reflex relations are recovered in orderly form and their reestablishment is strictly determined by anatomical factors regardless of functional suitability.

Effect of experience The tadpoles with rotated eyes were kept at least 1 month after recovery, four cases were retained nearly 2 months by which time the forelimbs had emerged. The reversed optokinetic reactions and cirrus locomotion persisted in all cases, and in no instance was any correction of the reversed responses noted.

EXPERIMENTS ON ADULT FROGS AND TOADS

Operations In 8 control cases (all bilateral) the optic nerves were sectioned without disturbance of the eyeball or ocular muscles. In 19 experimental cases (all unilateral) severance of the optic nerve was accompanied by rotation of the eyeball. The optic nerve was sectioned with jewelers' forceps through an incision in the roof of the mouth. The inner nerve sheath was completely severed in all cases, but in most animals, at least a connecting strand of the dural sheath was left intact. The eyeball was rotated by first severing all attachments to surrounding structures except the blood vessels and the optic nerve. This was done through two incisions, one in the roof of the mouth, the other around the outside of the cornea. The globe was then grasped by the stumps of the ocular muscles and rotated anterodorsally on its optic axis through 180 degrees. Some of the blood vessels were inevitably broken in the course of rotation, but a sufficient number remained to maintain circulation through the iris. The degree of rotation was estimated by distinct landmarks in the iris and pupil which varied in the different species. After it had been adjusted, the eyeball was allowed to set and heal in its new position after which the nerve of the rotated eye was sectioned as described. The appearance of the eye about 6 weeks after its rotation in four representative cases is illustrated in Fig. 1.

Vision in one eye is not easily tested in the presence of vision on the opposite side in anurans due to binocular overlap of the visual fields. Hence the contralateral optic nerve of the experimental group was sectioned either 3 days before rotating the eye (6 cases) or about 25 days after (13 cases). Thereafter the contralateral nerve was sectioned once or twice again after intervals of about 28 days whenever it became apparent that vision had been recovered in the unrotated eye.

tic axis When a fly was held in front of the animals within easy jumping distance, they wheeled rapidly to the rear instead of striking forward Contrariwise when the lure was held in back of them and a little to the side they struck forward into space When the animals came to rest in such a position that the lure could be presented well below eye level, they tilted the head upward and snapped at the air above When the lure was held above the head and a little caudad to the eye the animals struck downward in front of them and got a mouthful of mud and moss When the lure was presented successively in front of the animals they kept shifting around in circles as if the lure had appeared behind them each time instead of in front This brief description of the reversed reactions applies particularly to the responses of the specimens of *R. clamitans* and *R. pipiens* and also to the tree frogs when they were resting on the bottom of the terraria rather than clinging vertically to the walls The picture was of course somewhat different in the tree frogs when responding from a vertical position and in the toads, which struck mainly with a rapid flick of the tongue The essential reversal of the striking reactions, however, was clearly evident in all 14 cases As in the adult urodele, *Triturus*, and in the tadpoles the results in these adult anurans showed that the character of the recovered vision is determined systematically by intrinsic anatomical relations irrespective of the functional adequacy for the organism

There was a definite decrease in tendency to display the optokinetic response to movement of the visual field after eye rotation In 12 of the 14 cases optokinetic reactions could be elicited in some degree in preoperative tests but only four (1 *R. pipiens*, 1 *H. crucifer*, and 2 *H. squirrelle*) displayed discernible optokinetic reactions after recovery In 5 of the 14 cases the contralateral nerve had been sectioned 8 days prior to section of the nerve of the rotated eye in order to test in the interval the effect of eye rotation alone with the original nerve connections intact When tested all five showed nicely reversed striking responses, but only two (both *H. squirrelle*) showed any optokinetic reactions to rotation of the visual field In both these cases the responses were reversed and abnormally exaggerated Both animals also showed spontaneous turning movements of the head and body whenever they were aroused from their characteristic repose These movements were made toward the blind side and were thus comparable to the spontaneous circus movements displayed by the tadpoles and adult *Triturus* (8) after eye rotation After nerve regeneration in these 5 cases, the optokinetic responses were the same as before nerve section, i e., absent in 3 cases and reversed and abnormally exaggerated in the two *H. squirrelle* Apparently the failure in the majority of cases to get as good optokinetic reactions in reverse as were made in the correct direction preoperatively was thus correlated with eye rotation and not with optic nerve regeneration Since the spatial localization of small objects was a decidedly more critical test for the purpose of the present experiments, no further attempts were made through

normal position had no difficulty in locating and catching flies presented in any sector of the visual field. They struck with normal accuracy at the lure within the 15 cm range tested. Although attempts to approach the lure from distances as great as 40 cm were noticed a few times in some of the more aggressive animals, no systematic tests were conducted at these greater distances because of the great variability even among normal animals in tendency to respond. The animals made correctly directed preparatory turning movements of the head and body as the lure was moved about from one part of the visual field to another. That direction of movement was accurately perceived was further indicated by the fact that the more aggressive animals frequently struck and caught the lure as it was moving quite rapidly across the visual field in front of them.

The optokinetic response was also tested, but it proved to be rather variable in these adult anurans even before operation and not a very satisfactory index of visual recovery. Some animals showed quite good reactions while in others the response was barely discernible or lacking. Even the reactions of individual cases varied considerably from one test to another. In general the reactions were about the same in the control cases after recovery as they had been in preoperative tests. In the two animals which recovered on only one side, however, responses toward the seeing side were absent. The optokinetic reactions after recovery were always correctly correlated with the direction of rotation of the visual field. All tests indicated that the recovered vision in the control group was normal in character and not confusedly blurred as might have been expected if functional termination of the regenerating fibers in the visual centers had been random and non-selective.

It should perhaps be pointed out that the term "normal" is used in describing these results merely in a qualitative sense to distinguish normal from randomly confused vision and from reversed vision. It is quite possible that quantitatively the recovered vision was not up to normal standards. Visual acuity and intensity discrimination, for example, might well have been subnormal without noticeably affecting the animals' proficiency within the range covered by these tests. Crude tests of the size of moving objects at which the animals would strike, however, failed to reveal any significant difference between the operated and unoperated animals. Also, the term covers only the particular capacities involved in localizing small objects in space (in the adults) and in making correct optokinetic reactions (in the tadpoles). Form perception, such as it is in amphibians, color discrimination, if present (11), and any other aspect of visual function not involved in these tests may or may not have been normal in character after nerve regeneration. At the same time it must be recognized that the localization of objects in space is very probably in adult anurans the primary and most highly specialized function of vision.

Results with rotated eye. Localization of the lure by the experimental cases in which the eye had been rotated was from the start reversed about the op-

The anurans are exceptionally suitable for this type of test. Their tendency to remain in a set position for long intervals without any movement of head or eyes makes it easy to bring the test object into such position as to stimulate only the particular retinal area desired. The fact that responses to the lure are made primarily with the head and body without any appreciable independent exploratory movement of the eyeball also makes for clear-cut results. Walls' (11) statement, however, that "no amphibian is known to perform any eye movements other than retraction and elevation" is hardly accurate. Rotatory and turning movements of the eye in the orbit are pronounced and striking in the tadpole. Such movements are also common, though of less amplitude, in adult frogs and toads. These movements, however, seem to be associated primarily with vestibular reflexes, and if any initial exploratory movements of the eyeball occur independently of head and body movements, they are certainly so slight as to be negligible factors as far as the following experiments are concerned.

Operations. Tests were run first on 15 animals (1 *B. terrestris*, 5 *H. cinerea*, 7 *R. clamitans*, and 2 *R. pipiens*) with normal optic nerves. Lesions involving one half to two thirds of the entire lobe were made bilaterally in the anterior, dorsal and posterior portions of the lobe, each type of lesion being made in five animals. There was no attempt to produce lesions in the ventral portion, mainly because of the difficulty of avoiding injury to afferent and efferent fibers running to and from the other parts of the lobe. After the skin had been cut and reflected the cranium was broken away in small pieces and the outer meninges were cut or torn off until the dorsal aspect of the lobes was completely exposed. The lesions were begun with fine-pointed jewelers' forceps and completed by the suction method using a drawn-out point of glass tubing about 0.3 mm inside diameter. Animals were selected which were hungry and approached and struck readily at the lure when it was presented from any direction above, below, behind, or in front of them.

When tested within an hour after operation the five animals with the anterior part of the lobe intact made no response when the lure was shown in the back part of the visual field but struck vigorously and accurately when it was shown in front. The five animals in which the ventral part of the lobe remained intact made no response when the lure was presented anywhere in the visual field above them but turned or struck forward readily when it was presented below eye level. When the lure was presented behind the five cases in which the posterior part of the lobe was intact, they turned quickly so as to face the lure in preparation to strike just as do normal animals but, when they had thus turned and the lure was directly in front of them, they made no further response until it was again moved into the back part of the visual field. By repeating the performance these animals could be made to turn around in circles without ever striking at the lure although they came into a good striking position with each turn. These 15 cases were again tested on several occasions during the following three days with similar results.

That the retina normally is projected upon the optic lobe in an orderly manner with the retinal axes reversed in the tectum is clearly indicated by these effects of tectal lesions. The results confirm the conclusions regarding tectal termination of optic fibers in anurans reached by Stroer (10) on the basis of anatomical studies. Just how precise, that is, how close to a point-to-point correlation, the retinal projection is in these anurans cannot, of

adaptation of the testing apparatus and method to obtain more consistent elicitation of the optokinetic reflex. In the 4 cases which exhibited the optokinetic response after recovery, it was made in reverse.

Effect of experience After the reversed nature of the recovered vision had been ascertained, the animals were thereafter fed and tested only irregularly and no special attempt was made to determine the anurans' ability to correct reversed visual reactions by the learning process over an extended period of time. The following observations are therefore only suggestive, not conclusive.

All 14 of the adult cases with rotated eyes were kept at least 30 days after recovery of vision and four were kept longer than 70 days. Eight of the animals, never especially voracious feeders in the laboratory, showed no reliable change in their tendency to respond in reverse. Four cases, two of which had at first been quite aggressive in their misguided efforts to catch the lure, gradually became less responsive and finally refused to strike at the lure at all. After reaching this state, however, they also refused to eat even when flies were brought into direct contact with the nose, the regular method by which the animals with reversed vision were fed. It was thus not entirely clear in these four cases that the gradual decrease in frequency and vigor of reversed reactions was indicative of learning. Two other cases sacrificed at 78 and 83 days after recovery remained, on the other hand, particularly active and aggressive to the end in their misdirected attempts to catch the lure whenever it came in sight.

During the post-recovery period living flies placed periodically in the terraria with the animals with reversed vision remained uncaught except for the few that lighted or walked directly upon the animals, whereas they were immediately snatched up when placed in terraria with the animals with unrotated eyes which had recovered normal vision. The reversed optokinetic reactions and the spontaneous circus movements observed in the two *H. squirella* were still present at 40 days and 44 days after recovery when the animals were sacrificed. Thus in summary, there was a suggestion that the adult anuran may in some cases learn to inhibit the useless reversed reactions, but no indication in any case of a positive correction.

RETINAL PROJECTION ON THE OPTIC LOBE

In trying to determine how the central associations are formed in regeneration it becomes important to know whether fibers from any given retinal region have to make their way to a special localized area of the optic lobe in order to establish correct functional relations or whether it makes no difference in which region of the optic lobe the ingrowing fibers happen to terminate. If there exists an orderly projection of the retina upon the optic lobe, localized lesions therein should produce scotomas or blind spots in the visual field. Such blind spots should be detectable by noting the sectors of the visual field in which a lure can be held without eliciting any response, provided, of course, the animals respond readily when the lure is shifted into other sectors.

was sectioned longitudinally in different planes at 10μ . The findings in those cases in which visual recovery was unsuccessful have already been mentioned.

The point at which the optic nerve had been cut was recognizable, particularly in the adult animals, by an enlargement of the nerve trunk through which individual fibers and small fiber bundles took intertwined and tortuous routes. From the entangled appearance of the neuromatous scar region it was apparent that there had not been an orderly fiber outgrowth in bridging the gap between the distal and proximal nerve stumps. The systematic restoration of proper central linkages in spite of chaotic outgrowth across the nerve gap seems unaccountable except on the assumption that fibers from different retinal regions have specific inherent properties which influence selectively the formation of central associations.

Because of the small size and great number of fibers in the optic nerve of these anurans—Breusch and Arey (2) estimate 29,000 fibers in *R. pipiens*—the histological picture was too complicated to make possible any inferences regarding the question of whether or not the regenerating fibers attain an orderly segregation in the proximal stump or in the central tracts en route to the optic lobe. The results of tectal lesions do not settle the question because it is possible that the ingrowing fibers reach the optic lobe with a random distribution but only those succeed in establishing functional synaptic connections which happen by chance to enter their proper area.

Postmortem examination of the tectal lesions revealed that their general location was as intended, but there was considerable variation in border outline. The nature of the lesions and also of the behavioral tests did not permit any detailed quantitative comparisons of the precision of retinal projection in normal and regenerated nerves.

COMMENT

The foregoing data on recovery of vision after optic nerve regeneration in six different species of anuran amphibians support the previous conclusions based on visual recovery in the urodele *Triturus* (8). These conclusions and their logical basis are, briefly, as follows. Since stimulation of different retinal areas evokes different responses, each retinal locus must possess functional connections with the brain centers differing from those of all other areas. After optic nerve regeneration these differential relations between retina and visual centers are systematically restored in their original form, as shown by tests of optokinetic responses and visual localization of small objects. This orderly restoration of central reflex relations occurs regardless of the orientation of the retina, despite a maladaptive effect for the organism, and must therefore be regulated by growth factors independently of functional adaptation. As this would otherwise be impossible the ingrowing optic fibers must possess specific properties of some sort by which they are differentially distinguished in the centers according to their respective retinal origins. The previous discussion of these points with some of their implications (8) is in large part applicable to the present data and need not be repeated.

The orderly topographical arrangement of functional relations found in the optic lobe after optic nerve regeneration is difficult to explain without assuming that the secondary neurons of the optic tectum are also biochemically dissimilar, possessing differential affinities for fibers arising from different retinal quadrants. The results thus lend further support to the supposition that neurons of the central nervous system are specified biochemically in much greater degree than is evident from their morphological variations. A degree of central neuron specification is indicated which approaches

course, be deduced from the above results. The observations can be taken to indicate only grossly the existence of an orderly projection of the retinal field upon the optic lobe.

RETINAL PROJECTION AFTER NERVE REGENERATION

Since the redistribution of nerve fibers in regeneration has elsewhere been shown to be indiscriminate both in mammals (4) and in amphibians (14) the question arose as to whether the systematic projection of the optic fibers on the tectum might not be drastically disarranged after regeneration, necessitating a rather complete reorganization of secondary synaptic associations in the optic lobe. Accordingly, tectal lesions similar to the preceding were made in a group of animals which had recovered vision following complete severance and regeneration of the optic nerve.

Operations. Eight cases (1 *B terrestris*, 4 *H cinerea*, 1 *H squirrela*, 1 *R clamitans*, and 1 *R pipiens*) were selected which had recovered normal vision after optic nerve regeneration and were quite aggressive in their attempts to approach and snap at the lure when it was presented in any part of the visual field. Anterior lesions were made in 4 cases, posterior in 2 cases, and medial lesions in the remaining 2. Anterior lesions were stressed because the behavioral check is more strikingly conclusive. Under normal conditions an animal partially disinclined to strike at the lure will often not shift the head and body in order to get at the lure to the rear but will strike forward where the lure is easily accessible. Thus animals that would turn around to get at the lure—as they did after anterior lesions in the above cases—would certainly strike at the lure directly in front of them if it could be seen. Three of the eight cases were selected from the control group and the remaining five, all unilateral, were chosen from a group of extra cases prepared especially for the purpose.

The types of scotoma produced in these cases with regenerated optic nerves conformed consistently with those which had resulted in the foregoing group of normal animals. Posterior lesions abolished responses to the lure held behind, dorsal lesions to the lure held above, and anterior lesions to the lure held in front of the animals. Two additional cases (*B terrestris*, *H squirrela*) from the experimental group which had recovered reversed vision were also tested. A lesion in the anterior part of the optic lobe in one of them abolished responses to the lure presented behind the animal. When the lure was held in front, this animal responded by turning around to the rear in characteristic reversed manner. In the other case a medial tectal lesion abolished responses to the lure when it was held below eye level but did not eliminate the reversed reactions to the lure when it was presented well above eye level. From the nature of the scotomas produced by tectal lesions in these ten cases with regenerated optic nerves it may be concluded that the ingrowing optic fibers reestablish functional associations in the same topographical areas of the optic lobe in which they originally terminated and that no major reorganization of secondary synaptic relations is involved in the recovery of function.

ANATOMICAL CHECKS

All cases in which recovery of vision was absent or deficient, 2 tadpoles and 3 adults which recovered normal vision, and 3 tadpoles and 7 adults which recovered reversed vision were prepared for microscopic examination by the Bodian (1) method. The optic nerve

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whose retinas had been left in normal position but reversed about the optic axis in animals whose retinas had been rotated through 180 degrees prior to nerve section

3 The location of scotomas produced by localized lesions in the optic tectum after optic nerve regeneration indicated that optic fibers from different retinal loci had reestablished functional connections in the same areas of the optic lobe to which they had originally projected

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the degree of innate functional differentiation and which presumably plays an important role in the ontogenetic determination and differentiation of inherent integrating patterns

Where the functional properties within central nuclei have an orderly distribution corresponding with anatomical dimensions as in the anuran optic lobe, it is conceivable that a basic embryonic specification arises through central self-differentiation of the nuclear mass itself. Under such conditions the conjecture that specification of the tectal neurons may be induced via the more early differentiating motor and adjustor systems becomes unnecessary, although later acquisition of afferent and efferent relations may well result in further individuation superimposed upon the initial nuclear field.

The conclusion that function is not the organizing factor in the reestablishment of systematic reflex relations in these experiments in no way contradicts the possibility that function, as a generalized, non-specific factor, may be of importance for the normal healthy maintenance and development of nerve structures. That neuron discharge acts by itself in any specific manner, in optic nerve regeneration to regulate the formation of proper rather than improper or indiscriminate reflex associations, however, seems untenable. An inherent physico-chemical differentiation of the optic fibers must be inferred.

That such neuron differentiation is extremely important in the establishment of proper linkages between centers and peripheral end organs, in amphibians at least, becomes increasingly evident. Whether these linkages are determined primarily on a physiological basis involving specific modes of excitatory discharge and selective detection as envisaged in the resonance principle (13) or depend upon specific structural associations the formation of which is regulated through the influence of peripheral differentiation on central synaptic growth, as more recently proposed (6, 8), remains uncertain. The excitatory characteristics of mononeural connections between receptor and effector organs (12) and the fact that separate muscles which function asynchronously can be excited independently in their normal action phase by branches of a single motor axon (13, 14) seem more satisfactorily explained by the resonance principle. Until these latter phenomena have been more thoroughly studied, however, it seems advisable to stress at present the alternative possibility of interpreting effects of neuron specification on a more orthodox connectionist basis.

SUMMARY

- 1 In larval and adult anurans of six different species regeneration of the optic nerve resulted in a return of visual perception which was well organized, not an intermingled confusion. Distinct and consistent responses to position and direction of movement of objects in the visual field were recovered.

- 2 The orientation of visuomotor responses after recovery, however, was dependent upon the orientation of the retina. It was normal in animals

THE PERIPHERAL UNIT FOR PAIN

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VARIOUS TYPES OF sensory spots of the human skin can be individually and precisely stimulated by electric currents (8) Spots so identified appear to be identical to the sensory spots located by mechanical or other stimulation, while the electrical stimulus may be more accurately controlled, more sharply localized, and of shorter duration than other types of stimulus In a previous report a technique was described for controlled stimulation by means of electric spark discharges, without mechanical deformation of the skin, (2) and the sensory responses were noted to stimuli in various patterns of frequency and strength for touch and for pricking-pain endings The present work deals with the latter only, in an attempt to further define the structural and functional characteristics of what appear to be in some respects sensory units, but which are capable of inducing, under different patterns of stimulation, sensations of light contact, prick, itch or pain, all from the same minute locus

PROCEDURE

A first requirement was to isolate by some means an individual unit in the skin sensory mosaic Of what this is a unit, may be a matter of debate, but a unit of a sort can be isolated by anaesthesia of a nerve twig supplying a limited area of skin Advantage may be taken of the overlap between the distributions of adjacent twigs or of separate nerves supplying an area, a phenomenon well known If a small twig is blocked, at the periphery of its field of reference there will be found an occasional spot anaesthetized which is entirely surrounded by normally sensitive spots innervated by other twigs On the other hand, there will be an occasional spot near the center of the field of reference which will be normally sensitive, but surrounded by anaesthetized spots In the more fortunate cases a prick spot may be so located in an area where ordinary touch is also completely blocked, a coincidence involving two variables, the somewhat independently overlapping distributions of touch and prick After a number of trials, it appeared desirable to plot the nerve distribution over a considerable area of the author's right arm The courses of nerve twigs were located by strong stimuli through the skin, and areas to which sensations were referred were then mapped in detail

A repetitive stimulus of ten per second was employed, of a strength which may be evaluated functionally as follows The skin was shaved and thoroughly dried, and tests were carried out in a room at 75°F A few sensory spots were initially explored, and their most sensitive foci accurately marked with ink Between adjacent "high" spots prick could be felt almost anywhere if the stimulus were strong enough The strength was set to produce threshold prick sensation at a region about half way between prick foci, disregarding the fact that in some small areas no prick could be obtained at all with stimuli of any reasonable strength When this strength of repetitive shocks was applied to the more sensitive prick foci, sharp and persisting pain was felt These foci were marked as crosses with indelible ink Other foci gave definite prick at this strength which was not judged to be actually painful and these were marked with simple dots This same strength of stimulus was at about the lower limit of what would directly stimulate a nerve twig through the skin, and apparently only in those regions where such a twig lay unusually shallow A mild touch sensation was then referred to an area more or less remote Such a stimulus also excites touch endings at many points but this sensation can be readily differentiated from prick and ignored Prick in general has a lower electrical threshold than does touch (8),

*Aided by a grant from the Rockefeller Foundation for research in Neurophysiology.

After so mapping an area in the distribution of a nerve branch, 0.25 cc of 2 per cent novocaine was injected intra- or just sub-dermally along the course of the nerve. Prick was abolished usually within five minutes, and began to return within twenty minutes. Touch was abolished in over half the trials, for a maximum of ten minutes. Since the larger touch fibers in peripheral nerves are less easily blocked by novocaine than are pricking pain fibers (4), even partial abolition of touch indicates that all prick fibers in

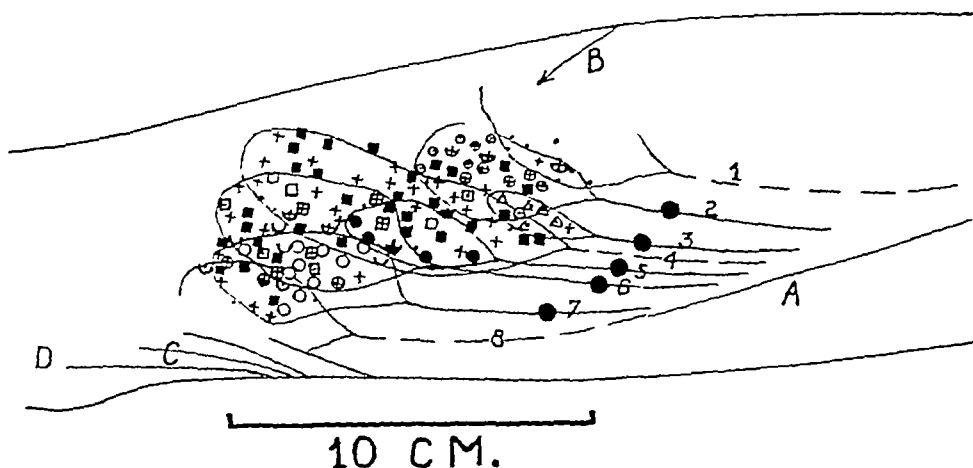


FIG 2 Area of dorsal surface of right forearm, innervated chiefly by branches of the dorsal antibrachial cutaneous nerve (A), and partly by medial antibrachial branches (B). At C the lateral antibrachial cutaneous sends branches across the back of wrist and hand to innervate the region just distal to that plotted here, and supplies the area of Fig 3 continuous with this figure. Pricking pain points were first plotted, the more sensitive as crosses, the less sensitive as dots, in areas of reference of sensation when nerve branches were stimulated electrically through the skin. The branches so located were then individually anaesthetized and the points whose stimulation no longer gave a sensation of prick or pain were encircled by symbols as follows: branches 1 and 8, inferred by presence of spots marked as dots, not otherwise accounted for; 2, open circles; 3, triangles; 4, dots and crosses; 5, solid circles; 6, open squares; 7, open circles. The remaining points, not blocked by anaesthesia of the main nerve at the elbow, marked as solid squares, are apparently innervated by branches of the medial cutaneous. Points whose sensitivity was reduced but not completely blocked by anaesthesia of a given branch are marked by a half of the symbol used for that branch. Many of these points were also partially blocked by anaesthesia of an adjacent branch, not indicated here, see Fig 3. Sites of injection of anaesthesia marked by large solid circles, nerve courses in full lines. Branches inferred but not found marked in dash lines. Reduced about one-half. Wrist joint at D, elbow joint just off figure to right.

that twig will have been completely blocked. After complete recovery the spots were again tested electrically.

Figure 2 is a composite diagram of distributions plotted on the dorsal surface of the right forearm. Branches of the dorsal antibrachial cutaneous 2, 3, 6 and 7 could be located by direct stimulation. Branch 5 was anesthetized by a random shot where a vacancy in the distribution of endings indicated its presence. Branch 4 is inferred from the fact that blocking of the whole nerve just below the elbow abolished sensitivity from a group of end-

although the least sensitive areas for prick on the forearm have a slightly higher threshold than the most sensitive touch spots. The most sensitive points for prick and touch are in general roughly staggered, and are rarely so closely adjacent as to be confusing.

Two features of this procedure indicate that nerve endings or end-organs rather than nerve axons are stimulated. First, the nerve fibers that serve prick in peripheral nerves have a higher threshold than do those for touch (3), the reverse of the order of the present results. Secondly, a strong stimulus to a nerve fiber, if brief, still gives rise to only one impulse, and previous work has indicated that this does not result in a sensation of prick. On the other hand a single brief shock to a prick focus gives a pricking or painful sensation which, at only moderately above threshold, noticeably persists.

SENSORY DISTRIBUTION OF NERVE BRANCHES

If now the stimulating needle is moved across the skin surface, just out of contact with the skin,¹ under a hand lens, and passing from one sensitive focus to another, the sensation fluctuates in intensity as the spark discharges trace a line across the skin. The result can be represented as a contour of intensity of sensation vs distance, as in Fig 1 A. The map of the skin, B, appears as a mosaic, of which the contour is in a sense a cross-section. Each cross or dot represents a minute and sharply located focus more sensitive than its immediate surroundings. The lines between and enclosing these foci in roughly hexagonal areas constitute the locus of minimal sensitivity, not necessarily of constant sensitivity, and not in general completely insensitive. The sharpness of the peaks in contour A gives a fair picture of the point-like character of the prick foci.

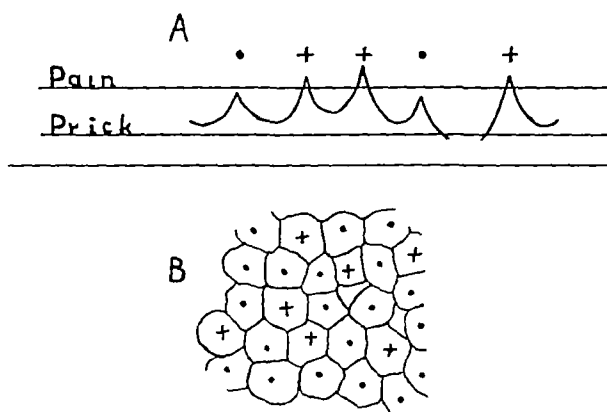


FIG 1 A Schematic plot of quantity of pricking pain sensation vs distance due to a constant stimulus, as the stimulating electrode is moved across the skin surface from one sensory high spot to another. Judgments of prick and pain are indicated by dots and crosses respectively above the peaks. B Schematic diagram of sensory areas of the skin, the central symbol of each area locating the most sensitive point, the lines dividing the areas being the loci of least sensation. In some small areas (no central symbol) no prick is aroused by even reasonably strong stimuli.

Each electrical mapping is checked over with a sharp needle point applied mechanically with too slight pressure to penetrate the skin. The location of foci by the two methods always checks, but the estimates of relative sensitivity of different foci may not. In cases of disagreement the results of electrical stimulation are those employed.

¹ While it is possible to so stimulate without touching the skin, routine experiments are easier to carry out if the needle rests on the skin surface. A number thirty-six B and S gauge wire 5 cm long in an insulated holder was employed, and on the shaved skin its contact or even motion cannot be felt, even as touch, until current flows. If hairs are present, such an instrument may bend them sufficiently to cause sensation.

possibility however that all of what are apparently unit cells in a mosaic are multiply innervated. The differences in sensitivity of the foci of different unit areas might be due to differences in number of fibers innervating them, but this also is conjectural.

It was stated that the unit sensory area that could be isolated extended about half way from a given blocked high point to adjacent points. Actually the anaesthetic area is slightly but consistently smaller than this. That is,

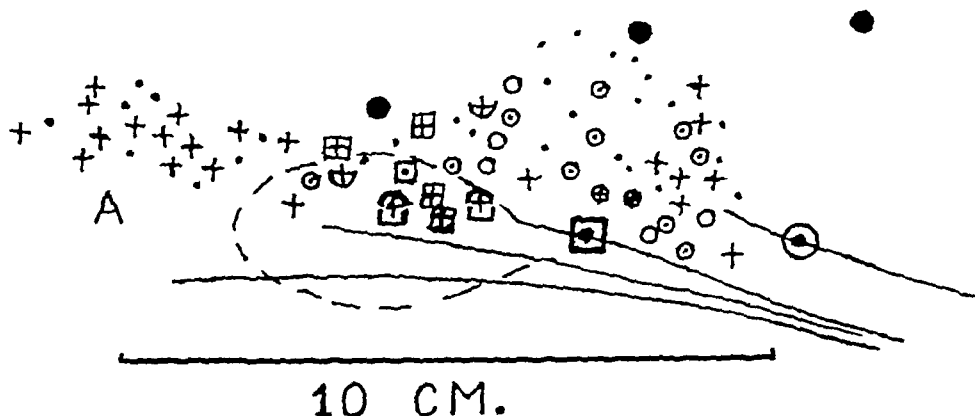


FIG 3 Two nerve block experiments on successive days, lateral surface of lower arm just above wrist, region on Fig 2 marked C. Symbols: large dot in circle, injection site, first day, needle inserted centrally along nerve for about 5 mm. Touch abolished over central area, diminished over rest of area where pain spots were blocked, dots, prick spots located before block, crosses, more severe pricking pain at strength of stimulus employed, circles about these symbols, spots completely anaesthetic after block, half circles, diminished sensation. Large dot in square, injection site second day, squares and half-squares, completely or partially blocked spots as above, area encircled by dash line, numb to touch. Crosses with half circle and half square, two spots of high initial sensation, partially anaesthetic after block of either nerve twig, and therefore presumably innervated by at least one fiber from each. Large dots above figure, india ink tattoo marks for reference and register of tracings of different experiments. Nerve twigs were located before injection by strong stimuli through the skin. The area of touch abolition encircled indicates that many more pain spots were affected by regions not explored, the margin between two twig distributions where multiple innervation might be detected was the object of investigation. The course of nerve branches is indicated in full lines.

the sensation from normal areas surrounding an anaesthetic unit decreases in intensity, with a constant stimulus, from each high point to slightly beyond half the distance to the blocked focus, where it can still be recognized as a definite prick. This must mean either that the electric stimulus spreads beneath the skin surface to stimulate endings a millimeter or more distant, or that the distributions of endings of adjacent areas overlap. In either case the result will be that a stimulus applied half way between two normal high points will activate the peripheral distributions of both of them. This overlap might be expected to result in summation, with an increased sensory effect from along this line of real or virtual overlap as compared to adjacent territory, but such is not found to be the case. This region of marginal

ings not previously reached. The more interesting of these branches have been blocked two or more times. Branches 1 and 8 are inferred from the distribution of points not otherwise accounted for. After block of the whole nerve, a fair distribution of spots over the whole area remained sensitive and were presumably innervated by a branch of the medial antibrachial cutaneous from the opposite direction. The expected branch could be located medially just below the elbow, and touch sensation from its stimulation was referred to the area previously plotted.

Pertinent features of this distribution pattern may be indicated. First, for the main branches in the arm, the distribution of each overlaps that of its neighbor at least half way, so that any small area may contain sensory spots innervated by each of three branches from the same nerve, and in addition by branches from an entirely different nerve. Second, the concentration of spots innervated from any one branch is greatest at the center of its distribution.

Character of a unit prick spot. After complete block of one nerve branch, the smallest area completely anaesthetized, and still completely bounded by sensitive areas, consists of one high point or focus, plus the area around it extending approximately half way to adjacent foci. Similarly, the smallest area still sensitive, but completely bounded by anaesthetic areas, is a similar unit. As noted above, such spots are found in the periphery and near the center respectively of the nerve's distribution, and their isolation must be due to overlap of fibers from one nerve into the distribution area of another (Fig. 2 and 3). A first inference might be that such a unit area comprises the distribution of the terminals of only one nerve fiber, or at least of one branch of one fiber, but this is certainly not true in some cases. The exception may be demonstrated by the presence, after thorough nerve block, of certain foci whose sensitivity is materially reduced, but definitely not abolished. These number not over one third of the number of foci from which sensation is completely abolished, and must indicate that a fiber from some other branch than the one blocked also innervates the locus in question. The estimate of whether the sensation is reduced by anaesthesia which does not abolish it is a precarious one for the less sensitive spots, but is fairly conclusive in the case of the more sensitive (marked by crosses), especially when equally sensitive spots in the immediate neighborhood remain normal, so that specific comparisons can be made during, before and after anaesthesia. A sensation which before or after block is definitely painful, becomes for such partially blocked spots a non-painful but still definite prick, approximately like that from the unaesthetized spots designated as dots on the chart. In a number of cases the block of either of two nerves has diminished the response from a given spot.

If two fibers from two different nerve branches may thus innervate an area which has certain unitary characteristics, it is highly probable that two or more fibers from the same branch will do the same, and perhaps more often. The present technique is inadequate to test the latter case. This raises the

sensation caused by the first, that is, the first sensation completely masked the second. To the other half, application of the second stimulator resulted in pronounced enhancement of the sensation, that is, the two summated as one locus. Testing the next orbit, of points one removed from a given point, none showed masking, a few again gave summation with the first without discrimination as two points. The rest gave sensations as from two recognizably discrete points, but *without significant summation of intensity*. Results were similar on the back of the forearm and on the front of the thigh, the difference being chiefly in the greater distance apart of sensory points on the thigh. The above results follow from strong stimuli causing definite pain. Stimulation of points still farther removed from each other were discriminated as two points, with slight if any summation.

The same procedure was carried out with alternate bursts of stimulation from the two needles. When applied to adjacent high points, of course neither summation nor masking were to be expected, since at any given moment only one needle was delivering stimuli. The criterion here was a pulsating sensation, most pronounced when one needle only was applied to one spot. This sensation then built up by temporal summation during each one-second burst, and persisted with a decremental intensity throughout the following one-second intermission. Now when the second needle was applied to any one of the six adjacent high points of comparable sensitivity, the pulsation promptly ceased, and the sensation became perfectly steady, and was indistinguishable from the result of application of both needles to the same point. This result also followed application of the second needle to some of the points once removed from the test point. For other points in the second orbit, and for any point farther removed, the sensation could be felt to pulsate and to alternate between two discrete points that could be discriminated.

When any two points are stimulated alternately, and still no pulsations are felt, either the two points must be identically innervated, or their pathways must converge to give smooth temporal summation above the first sensory neurone. It will appear below that this convergence is affected by the intensity of the stimulus. In fact there appears to be a certain incompatibility between two-point discrimination and spatial summation, any two points whose equal stimulation causes material summation are not recognized as separate points, and any two points recognized as separate do not show significant summation, under a given set of conditions.

The picture may be elaborated somewhat by the results of the same procedure with weaker stimulation, or with any stimulation at lower frequency, down to one or two shocks in one-second intervals. The less effective the stimulation, the farther apart two stimulated points must be, to be recognized as two separate foci. As in many other sensory situations, visual acuity for instance, discrimination is two-dimensional, involving parameters both of space and of quantity of stimulation, reciprocally related. Points which seem discrete with strong stimulation and show little summation, with

overlap gives the least sensory response of any. The presence of maximal sensitivity at the center of the distribution on the other hand checks with the findings of Tower for the cornea (6) where the center of a given fiber's distribution was the most sensitive region for mechanical stimulation, as indicated by the maximal frequency of the single fiber's response.

Prick localization and interaction between adjacent unit areas Localization and two-point discrimination are much less precise for prick or pain than for touch. Under electrical stimulation, a change of locus is recognized for touch as the repetitive stimulus is moved across the border between adjacent unitary touch areas (2). This is not true for prick. To test the matter, several stimulating needles were connected through as many condensers and auxiliary apparatus (Fig. 4), in the stimulating apparatus previously described (2), all operated synchronously by the shocks from the same thyatron stimulator. A needle could then be applied to each of several sensory spots. To avoid complications of adaptation and fatigue, a circuit to ground was connected

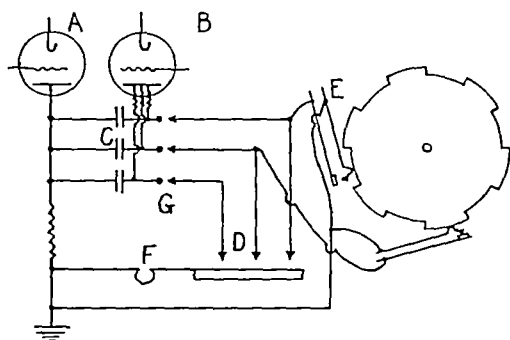


FIG. 4 Details added to apparatus previously described to permit simultaneous stimulation of more than one point of the skin. A and B are vacuum tubes delivering pulses and steady current respectively, the latter serving to discharge the condensers C between pulses and keep the air gaps at G ionized for constancy of operation. Stimulating needles at D are applied to the skin, with phones F between body and ground. A short circuit through the circuit-breaker E is opened periodically by a key, one to each circuit, whereupon a burst of stimuli is delivered by the corresponding needle. The setting of keys indicated gives alternate bursts from two needles.

to each of these needles, shunting the body, and each ground circuit could be opened by means of a key on a rotating circuit-breaker, whereupon a burst of stimuli from the needle of that circuit was delivered to its sensory spot for a predetermined period. The most readily interpretable results were obtained by the use of two needles, with stimulation of about ten per second, one second on and one second off. The "on" periods for the two needles could be made either to coincide, or to alternate.

With stimuli strong enough to summate to frank pain over one-second periods, repetitive bursts of repetitive stimulation induced a pulsating sensation that was easily evaluated. With synchronous bursts from two needles placed on the most sensitive points of adjacent areas, the two points could not be discriminated as separate points. If one of the needles was now lifted off the skin periodically, one of two results was obtained. Each unit area is surrounded on the average by six contiguous areas. To about half of these latter, the application of the second stimulator induced no change in the

not a novelty peculiar to the present technique, in a tissue where its anatomical substrate appears to be meshwork of interwoven and overlapping plexuses of nerve terminals (10, 9) This problem has been raised before, and recently by Tower (6, 7) who pointed out that histological techniques are more capable of revealing possible complexities of distribution than they are of defining the actual limits of any one fiber field Physiologically several experimenters have determined such fields in animals (1, 6, 5) by single nerve fiber recording, without arriving at a sensory distribution that would be strictly punctate However, neither the cornea, nor so far as known the areas of animal skin so far explored, are known to contain such circumscribed loci of high sensitivity as are exhibited in the human skin over areas most liable to external contact

The finding of a "high" point of extreme sensitivity, surrounded by an area of decreasing sensitivity, may represent only an extreme case of the higher irritability of the central region of the fiber field of the cornea observed by Tower The overlap of fiber fields in the cornea might correspond to the multiple innervation of one sensory spot in the skin of the arm for which evidence was presented above, except that in this case complete superposition must be inferred as compared to the staggered overlap in the cornea, as a refinement perhaps for a more precise sensory localization

Certain functional aspects of dolorous sensibility certainly involve central interpretation, but also connote specific modes of peripheral action that correspond For instance, different degrees of stimulation (*i e*, different rates and durations of discharge of afferent fibers) result in sensations that differ qualitatively as well as quantitatively In addition to the slight and inconsequential touch that may be detected below threshold for prick, the sensations of prick, itch and pain that can be obtained from a given point become only the more qualitatively different as they are experienced under more controlled and specific conditions Itch does not merge into prick, prick does not become in itself painful when stimulus intensity is raised In the estimate of this observer, a new and different sensation is added It is as if increased frequency of fiber response registered not only as increased intensity of sensation, but at certain levels, switched in a different central recording system

A second consideration suggesting the same thing is the mutual exclusion, with reference to two given loci, of summation of intensity and two-point discrimination This again suggests a shift in central connections depending on quantity of peripheral excitation When such a subjective shift follows change in stimulus intensity, an increase in number of endings activated might be the factor assigned to account for it, but when the shift follows change in frequency of shock stimulation alone, at constant intensity, it must connote a qualitative central reaction to a quantitative peripheral change To attempt a more objective interpretation of so subjective a matter as sensory estimates must wait upon a more objective interpretation of the sensory mechanism itself than prevails at present

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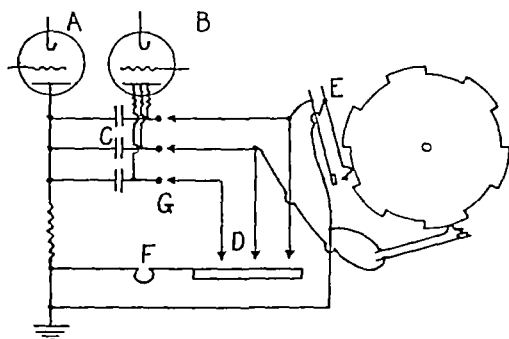


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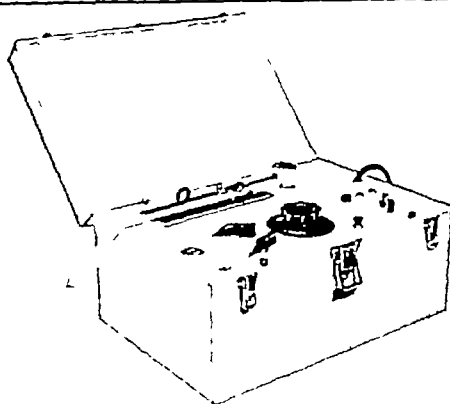
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weaker stimuli now summate as one subjective point of increased sensory response. The more striking effect follows alternate stimulation of two points. In general two such points will have somewhat different sensitivities, the effect of which is less noticeable with very strong stimuli. With weak stimuli alternately applied to distant points, the sensation may be one of alternate weak and strong sensations from the same point, which is localized as the more sensitive of the two even when discrimination between them is impossible. When the periods of stimulation are synchronous rather than alternate, the removal and reapplication of either needle gives a decrease and increase of sensation by summation, but referred to the more sensitive point. With weak stimulation at a slow rate, still effective as inducing definite sharp prick, points as far apart as four or five cm., on the front of the thigh where high spots are seven to ten mm. apart, may give summation as one point. With strong and higher frequency stimulation two points within fifteen mm. may be discriminated as separate.

Possible contributions of touch endings to localization of pricking pain. Since the electrical threshold for prick endings is lower than that for touch endings in general, the latter do not complicate an experiment involving threshold stimulation of prick. Shocks sufficient to cause pain may also arouse non-painful touch, and since touch is more closely localized than pain, it may be questioned why the results presented above are not matters of touch localization and discrimination, rather than of prick or pain.

The differentiation of touch from prick is materially aided by the different time course of sensory response to touch and prick respectively, originally emphasized by Von Frey (8). Touch is dead beat, and gives a momentary sensation to each shock, like a light tap. Prick or pain is definitely persistent, and in fact rises gradually to its maximum after each shock. A 10-per-second sequence of touch stimuli is not summated, while pain rises to a summated maximum only after five or six stimuli, and persists for the greater part of a second after the stimulus ceases. Ten-per-second is therefore effectively continuous stimulation for pain, and only intermittent stimulation for touch, and persistence of sensation facilitates localization or discrimination. Second, touch and pain high spots are generally staggered, and if the stimulus is delivered precisely at the most sensitive point for pain, touch will generally not be aroused. Finally, if touch sensations were being employed to localize or discriminate between points the results would show better discrimination than is found, as can be easily demonstrated by shifting the positions of the needles to touch loci.

DISCUSSION

One realizes at once that the sensory "unit" as here outlined does not correspond precisely to any anatomical description of nerve ending distribution to which the sensory modality of pain has been assigned. It would be futile to discuss here whether sensory terminals have been assigned erroneously, when so many other possibilities of interpretation exist. Rather one may ask how we can account for punctate pain distribution at all, certainly

INTERACTION OF NEIGHBORING FIBRES IN MYELINATED NERVE

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INTRODUCTION

DURING the past few years a vast amount of literature has been accumulated on the problem of the effect produced by the action currents of an active nerve fibre upon neighboring nerve fibres. The observations that have been reported on the basis of experimental work may be grouped in three categories: (i) Impulses traveling in a fibre or in a group of fibres may act as liminal stimuli and set up new impulses in neighboring fibres. Into this group belong, among others, observations made by Hering (14), von Uexküll (31), Kwassow and Naumenko (20), Jasper and Monnier (15), Arvanitaki (2, 3), Rosenblueth (30) and Renshaw and Therman (29). (ii) The passage of impulses along a fibre or a group of fibres may modify the electric excitability of the neighboring fibres. Measurements of the excitability changes have been made by Otani (27), Katz and Schmitt (17, 18), Blair and Erlanger (7) and Renshaw and Therman (29). (iii) Finally, there are observations on the modification of activity attributable to the interaction of neighboring fibres: synchronization of spontaneous rhythmic firing (1), modification in the speed of conduction of impulses (17).

All the observations of the first group are in satisfactory agreement, but considerable discrepancies exist in those of the second group. This paper presents the results of a series of experiments conducted with the purpose in view of clarifying some of the controversial points.

TECHNIQUE

The experiments were performed on the sciatic nerve of the bullfrog, using the arrangement of stimulating and recording electrodes indicated in the diagrams accompanying Fig. 2 to 6. A conditioning shock approximately two-thirds maximal α was delivered, for example, to the lateral peroneal nerve, and the changes in the excitability of the fibers of the medial peroneal nerve induced by the traveling impulses were measured in terms of the height of the response to a testing submaximal shock delivered through electrodes placed on the trunk of the sciatic nerve or on the medial peroneal nerve itself. In view of the argument that was presented by Graham and Lorente de Nó (13) and by von Brücke, Early and Forbes (8), since the observed changes in the height of the testing response were relatively small, they may be regarded as being proportional to the changes in excitability of the individual fibres. In the experiment illustrated by Fig. 2 the percentual change of excitability was determined by means of a calibration curve (spike height plotted against peak voltage of the testing shock).

To prevent distortion of the action currents of the conditioning volley of impulses, the coil delivering the testing shock was connected to the nerve in series with a large resistance. However, no significant difference in the interaction curves was found when this resistance was removed.

THEORETICAL ARGUMENT

An understanding of how the flow of the action currents of a nerve fibre may affect neighboring fibres can be obtained by an extension of the argu-

SUMMARY

1 A unit of a sort for pricking pain can be isolated in the skin by anaesthesia of a nerve branch, after locating by electrical stimulation all points of maximal sensitivity in the nerve's distribution. Taking advantage of the scattering overlap between adjacent nerve branches, the smallest area remaining unanaesthetized after block of one branch, but completely bounded by anaesthetized areas, consists of one highly sensitive point surrounded by an area decrementing in sensitivity toward its periphery. The smallest area anaesthetized but bounded by sensitive areas is a similar unit.

2 These units overlap slightly, but the marginal region of overlap is the region of minimal sensitivity. Certain units appear to be innervated by each of two nerve branches, and more are probably multiply innervated by axons from the same branch.

3 If two such units are stimulated coincidentally or by alternate bursts, two-point discrimination between them then depends on degree of stimulation, the greater the stimulation, in terms of either strength or frequency, the closer together are two points recognizable as discrete. Two points discriminated as such do not summate in painful sensation, and vice versa. Some adjacent points mask each other, i.e. they neither summate in intensity of sensation nor are they recognized as separate spots.

4 Itch, non-painful prick and pain, elicited by appropriate patterns of stimulation from the same point, differ in quality as well as quantitatively.

5 This qualitative shift, with quantity of stimulation, and the shift from summation to two-point discrimination similarly induced, point to a central qualitative interpretation of sensory impulses depending only on quantitative factors involving identical peripheral mechanisms within the single modality of pricking pain.

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C and the testing nerve *T* first come in contact, will be called the *fork*. The diagram shows that part of the polarizing current flows through the fibres of *T* in much the same manner as would the current from an ordinary stimulating circuit. By analogy it may be stated that in the membranes of the fibres of *T* there are "anodal" zones in which the current flows inwards, and "cathodal" zones in which it flows outwards. It must therefore be expected that the excitability of the *T* fibres, when tested by an electrical stimulus, will appear to be enhanced in the cathodal and depressed in the anodal zones. A significant detail in diagram *III* of Fig. 1 is the spread of the current of nerve *C* into the region of nerve *T* beyond the fork.

The argument applies in a similar fashion to the case of the action currents of a traveling impulse (Fig. 1, *IV*). Since fibre *T* is a part of the external conductor of fibre *C*, a fraction of the action currents of *C* must cross the membrane of *T* and in so doing will produce changes in the excitability of fibre *T*.

In Fig. 1, *IV* an impulse is supposed to be traveling in fibre *C* in the direction of the arrow, 1, 2 and 3 indicate the successive phases of the membrane current of fibre *C*, outwards, inwards, and outwards, respectively. R_1 and R_2 are the points at which the membrane current reverses its direction, and Cr denotes the position of the crest of the action potential. Since in fibre *T* the membrane current flows in a direction opposite to that in *C*, the passage of the impulse along *C* will successively produce an anodal, a cathodal and an anodal flow of current through the membrane of *T*.

The expectation, therefore, is that during the flow of the action currents of *C* the electric excitability of fibre *T* will pass through three successive phases: depression, enhancement, and depression, respectively.

EXPERIMENTAL ANALYSIS

The standard interaction curve. The argument above is in agreement with that used by Katz and Schmitt (17). It applies to the case of testing electrodes at some distance from the fork.

Figure 2 presents curves of the temporal course of the interaction obtained in an experiment that is typical for a series of similar experiments (cf. Fig. 5, *I* and 6, *I*). The experimental data (heights of the testing responses) are given upon photographs of the action currents of the conditioning fibres. These records were obtained by connecting the oscillograph to the testing cathode and to another electrode making contact with the nerve at a point 3 mm. beyond the cathode. The stimulating coil was removed from the circuit. As is well known, the height of the diphasic records obtained in this manner is proportional to the instantaneous value of the longitudinal current, that is to say the oscillographic deflection is proportional to the first derivative with respect to the length coordinate of the changes in the membrane potential (action potential). Since the membrane current is proportional to the second derivative of the action potential with respect to the length coordinate, the crests of the records reproduced in Fig. 2

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correspond to the reversal points (R_1 , R_2 , Fig 1, IV) of the membrane current, while the instant at which the deflection changes its sign corresponds to the crest (Cr, Fig 1, IV) of the action potential. From the records reproduced in Fig 2 it is obvious that, because the conditioning shock was adjusted to give an approximately two-thirds maximal α response, the traveling spike was practically synchronous (*cf* 10)

The interaction curves do show the three phases predicted by the theory, but their temporal relations do not agree in a satisfactory manner with the records of the action currents. In the first place a change in excitability is already observable when the testing shock is delivered before the flow of the action current at the level of the stimulating cathode becomes appreciable. Secondly, the third phase (depression) has its crest at a time when the flow of the action currents is very small, and this phase continues long after the cessation of flow of the action currents of the conditioning spike. Thirdly, the reversal points of the interaction curve do not coincide exactly with the reversal points of the membrane current. Fourthly, no quantitative agreement can be said to exist even during the first two phases of the interaction curve, because the change in excitability is not proportional to the slope of the diphasic records reproduced in Fig 2. Similar discrepancies between the flow of membrane current in the conditioning fibres and the interaction curve are found in the data of Katz and Schmitt (17).

The discrepancies between the temporal course of the action currents in the conditioning fibres and that of the excitability changes of the testing fibres are significant, and they indicate that the theoretical argument given above needs important elaboration. But the discrepancies are certainly not sufficient to justify the assumption that the agents responsible for the excitability changes are any other than the action currents of the traveling impulses. Moreover, the existence of three phases in the standard interaction curve rules out the alternative explanation of the mechanism of interaction that was discussed by Blair and Erlanger (7).

Blair and Erlanger (7), when working with the testing cathode at some distance from the killed end, obtained interaction curves consisting in the main of a single phase of depressed excitability, and since a curve of this type roughly parallels the decrease in the transverse resistance of the membrane during passage of the impulse that was observed by Cole and Curtis (9) in working with the giant axon of the squid, Blair and Erlanger considered the possibility that, when the resistance of the membrane of the active fibres is decreased, these fibres become a shunt for the testing current and reduce its effectiveness as a stimulus for the inactive fibres.

Katz and Schmitt (18) suggested that changes in the resistance of the membrane of the active fibres could not be a significant factor, because the shape of the interaction curve is independent of the position of the testing anode. However, although the experimental fact is true (*cf* Fig 2), the argument cannot be said to have general validity. For example, under the conditions of the experiment illustrated by Fig 2, a change in the resistance of

ment used by Helmholtz, Hermann, etc (cf review by Biedermann, 4) in their analysis of the spread of electrotonic currents from a nerve that is being polarized to a second nerve in longitudinal contact with the first

Figure 1, I reproduces the classical diagram of the electrotonic spread of a polarizing current applied to the nerve through electrodes of negligible width placed on its surface For the sake of simplification, the nerve is supposed to contain only one fibre Theoretically the current spreads through the whole nerve, but since the electrotonic membrane potential has an approximately exponential decrement, with polarizing currents near rheobase

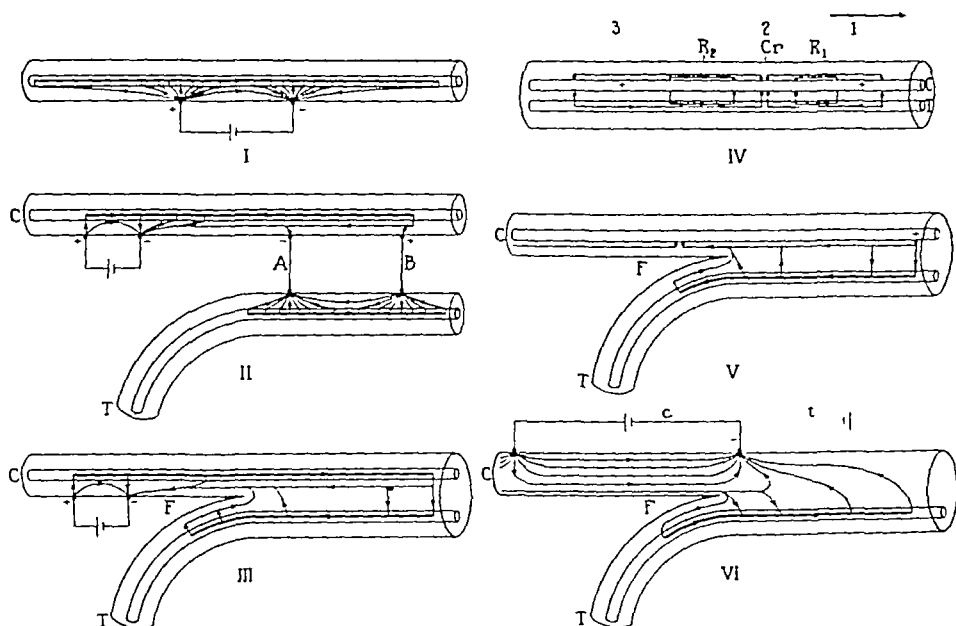


FIG 1 Diagrams to explain the mechanism of the interaction of neighboring fibres *C*, conditioning, *T*, tested fibres, *F*, fork The arrow above diagram IV indicates the direction of travel of the conditioning impulses Further details in text

the spread becomes undetectable at distances 15 to 20 mm beyond the polarizing electrodes

It will be seen in the diagram that electrotonic spread results in a longitudinal flow of current in the external conductor of the polarized fibres The ohmic drops of potential thus produced can be used to polarize a second nerve For example, if the directly polarized nerve *C* is connected with a second nerve by two wires (Fig 1, II, *A* and *B*), there will be found in the second nerve *T* a distribution of currents which, except for the direction of flow, is similar to that in nerve *C*

The situation is identical in principle though differing in detail if, instead of establishing electrical contact by means of wires *A* and *B*, nerve *T* is placed in direct contact with nerve *C* in the manner indicated by diagram III of Fig 1 For convenience the point *F*, at which the conditioning nerve

fibres must always have the same direction as the longitudinal current in the external conductor of the *C* fibres. Diagram V of Fig 1 shows that at the fork an outward (cathodal) flow of membrane current must be established in fibres *T* as soon as the flow of action currents of *C* reaches the common trunk. The outward flow through the membrane of *T* will continue until the crest of the action potential of *C* reaches the fork. Thereafter the current through the membrane of *T* will have the opposite direction. It is therefore clear that at the fork the curve of immediate interaction can have only two phases, enhancement and depression, corresponding to the two phases (ascending and descending) of the action potential of the *C* impulses, or the two phases (negative and positive) of its derivative with respect to the length coordinate. Moreover, since the flow of current spreads electrotonically ahead of the fork into nerve *T*, excitability changes should also appear in this segment of *T*.

Experimentation has confirmed both predictions. In Fig 3, curve *I* presents the excitability curve obtained with the testing cathode 1 mm from the fork, and curve *II* the excitability curve obtained with the testing electrodes ahead of the fork, the cathode being approximately 1 mm from the latter. As was expected, the initial phase of depression is lacking in the two curves.

A variation of the experiment is illustrated by curves *III* and *IV* of Fig 3. Curve *III* was obtained with the testing cathode 35 mm from the fork. As a proof that the effect of the fork extends that far it will be seen that curve *III* also fails to exhibit the initial phase of depression. An artificial fork was created 8.5 mm from the testing cathode by placing between the two branches of the nerve a pledget of cotton soaked with Ringer's solution. The result was the appearance of the initial phase of depression of the standard curve, because the application of the cotton pledget modified the conditions for the flow of

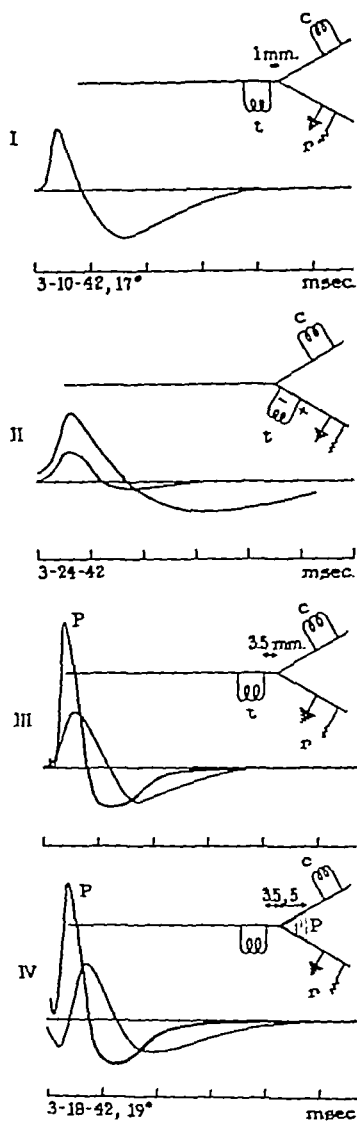


FIG 3 Interaction curves obtained in the neighborhood of the fork *P*, tracings of records of the difference of potential established between testing cathode and anode by the conditioning volley of impulses. In II the lower curve is the recorded potential change.

the conditions for the flow of

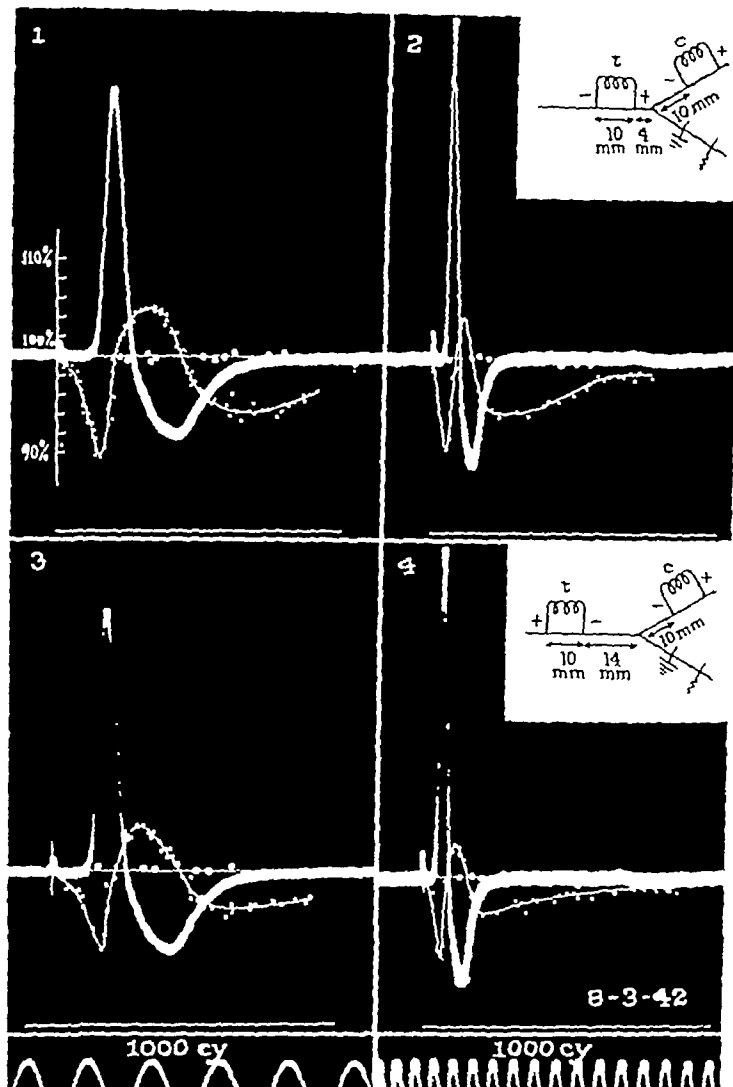


FIG 2 Standard interaction curves superimposed upon records of the longitudinal flow of the action currents of the conditioning fibres. The arrangements of conditioning (c), testing (t) and recording electrodes used to obtain the interaction curves are indicated in the diagrams next to records 2 and 4. Records 1 to 4 were obtained with recording electrodes 3 mm apart at the level of the testing cathode, the amplification being slightly larger for records 2 and 4 than for records 1 and 3. Records 1 and 2 are retouched and 3 and 4 untouched prints from the original films.

The height of the unconditioned testing response is given by the distance between the base lines at the bottom of the records and the horizontal lines (circles) through the records. The dots give the heights of the conditioned responses. Distances on the abscissa axis measure the interval between the conditioning and the testing shocks. The scale in record 1 measures the excitability changes in percentages of the resting excitability.

diagram VI of Fig 1 In the case of curves *Ia* and *Ib*, the conditioning shock was above threshold and the effect of the traveling impulses followed that which had been produced by the shock itself According to the argument discussed in reference to Fig 1, IV and V, the effect of the traveling impulses should begin with a phase of facilitation, and this effect was actually observed, superimposed on the effects of the shock itself The effect appeared later in the case of Fig 4, *Ib*, because the impulses were obliged to travel through a longer segment of nerve from the conditioning cathode to the fork The reason why the excitability changes also were observed when the conditioning shock was preceded by the testing one (Curves I to III) is

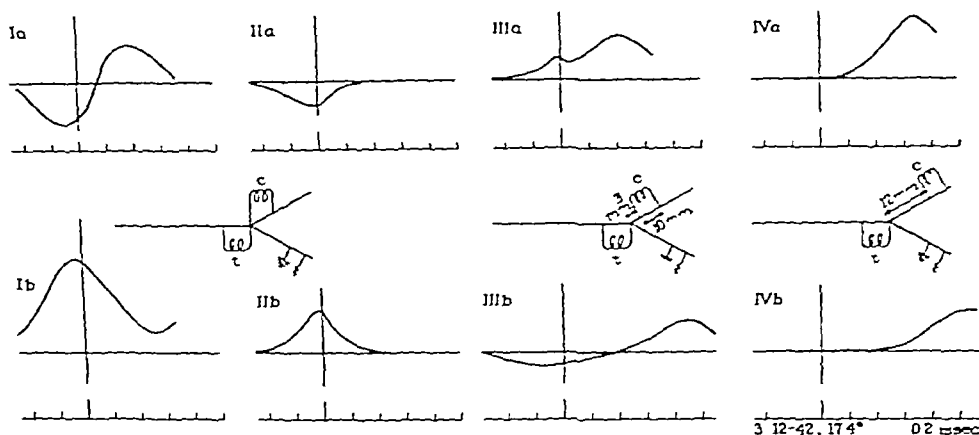


FIG 4 I and II, interaction curves obtained with an electrode at the fork which was common to the conditioning and the testing circuits *Ia*, *IIa*, conditioning shock cathodal, *Ib*, *IIb*, conditioning shock anodal *Ia*, *Ib*, conditioning shock submaximal, *IIa*, *IIb*, conditioning shock subliminal. Curves III and IV illustrate the effect of displacing the conditioning electrodes away from the fork (conditioning shock submaximal) *IIIa* and *IVa*, conditioning shock cathodal, *IIIb* and *IVb*, conditioning shock anodal.

essentially the same reason that will be given in the next section to explain the early start of the standard interaction curve

The peculiar situation at the fork illustrated by diagram VI of Fig 1 of course disappears when the conditioning electrode is moved away from the fork Then the current of the stimulating shock, while spreading electrotonically along the C fibres, produces in fibres T a flow of membrane current in the same manner as that produced by traveling impulses (Fig 1, V) For this reason in Fig 4, *IIIa* and *IIIb*, the cathodal conditioning shock enhanced (*IIIa*) and the anodal depressed (*IIIb*) the excitability of the tested nerve fibres When the conditioning electrodes were moved farther away from the fork, the spread of the current of the conditioning shock to the fork was too small to produce any noticeable change of excitability, and consequently the tests demonstrated only the effect which is referable to the action currents of the traveling impulses (Fig 4, *IVa* and *IVb*)

the membrane of the active fibres at the level of the testing anode cannot have any measurable influence upon the flow of the testing current at the level of the cathode, because the testing electrodes were 10 mm apart and the secondary coil, which was shunted by a 10,000 ohms resistance, was connected to the nerve in series with a 500,000 ohms resistance. Under conditions such as these it follows from the theory of the core conductor* that the flow of current through the cathodal segment of the nerve is independent of happenings at the anode.

Without attempting to explain in detail why the interaction curves of Blair and Erlanger deviate from the standard curve,** it may be said that the resistance-change hypothesis cannot explain the existence of three phases in the curve, nor can it meet a serious theoretical objection, namely that since the decrease in the transverse resistance of the membrane is accompanied by a change in the E M F, the apparent resistance that the nerve fibre offers to the passage of an externally applied cathodal current does not necessarily need to decrease when the impulse, that is to say, the wave of depolarization, reaches the cathode. Actually it must increase, because (1) according to unpublished observations made by Lorente de Nó, in frog nerve the decrease of the transverse resistance of the membrane during passage of the impulse amounts to only a few per cent of its resting value, and (2) the density of the liminal cathodal current is so small that it produces across the membrane a potential difference which is of the order of magnitude of the resting E M F. Thus for the interpretation of the interaction curve the significant factor is the change in the E M F of the membrane, or rather the flow of action currents that it causes.

ELABORATION OF THE THEORETICAL ARGUMENT

The interaction curve at the level of the fork. Before elaborating the theoretical argument to account for the details of the interaction curves, it will be convenient to consider the curve that is obtained when the testing cathode is placed in the neighborhood of the fork.

Diagram V of Fig. 1 indicates that when impulses are traveling along the C fibres, a singular situation appears in the neighborhood of the fork. It cannot be expected that in this segment of the tested fibres the flow of membrane current will at all times agree with the direction of flow through the membrane of the C fibres, because at the fork the membrane current of the T

* A comprehensive discussion of the theory of the core conductor is given by one of us (R. Lorente de Nó) and L. Davis, Jr., in a forthcoming monograph.

** The experimental conditions used by Blair and Erlanger are rather complex. In our experience, curves of the type described by Blair and Erlanger are obtained when the conditioning volley of impulses, instead of being a fairly synchronous two-thirds α spike, is a maximal spike with considerable dispersion after long conduction. Another condition that results in distorted interaction curves is the superposition of the effects of (1) the shock initiating the conditioning volley and (2) the action currents of the conditioning impulses themselves (cf. Fig. 1, VI, and 4).

plots indicates that the conditioning and the testing shocks were delivered simultaneously. Record *P* of Fig 5 gives the time of arrival of the traveling impulses when the conditioning shock was delivered at zero time. The inter-

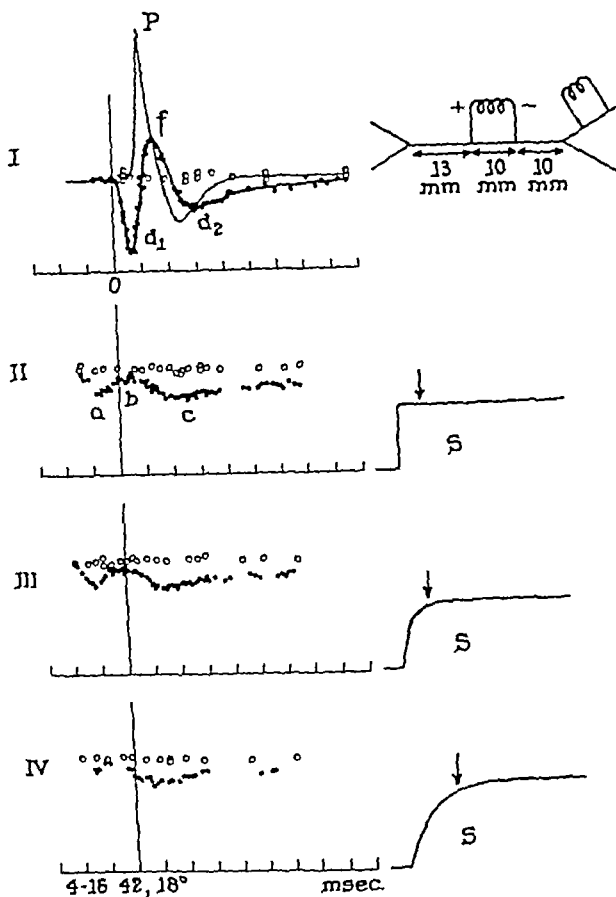


FIG 5 Modifications of the standard interaction curve resulting from a change in the testing stimulus. For curve *I* the testing stimulus was an induction shock, for curve *II* a rectangular pulse of current, and for curves *III* and *IV* exponential pulses. *P*, difference of potential established by the volley of conditioning impulses between the testing cathode and the testing anode. *S*, records of the electrotonic potentials at the testing cathode established by subliminal pulses of current of the same shape as the submaximal pulses that were used as testing stimuli to obtain curves *II*, *III* and *IV*. The arrows indicate the end of the utilization period of the submaximal pulses.

vals of time between shocks are regarded as positive and plotted to the right of the zero point when the conditioning shock (shock to the C branch) was delivered first, but they are regarded as negative and plotted to the left of the zero point when the "testing" shock (shock to the common trunk) preceded the "conditioning" one. The utilization times of the pulses of current

action currents and was equivalent to a displacement of the testing cathode along the nerve trunk, away from the fork

Up to this moment, in developing the theoretical argument, the nerve as a whole has been regarded as a linear conductor, that is to say one in which longitudinal currents have uniform density throughout the cross section of the nerve. However, in a strict sense, nerve is not a linear conductor, because it is a cylinder having a finite radius, which is suddenly increased at the fork

In a qualitative analysis of the electrotonic spread of current along the nerve fibres or of the spread of the action currents of one fibre into neighboring fibres, it is hardly necessary to take into account that nerve is not a linear conductor, because the electrotonic spread is due to the core-conductor properties of the individual fibres, and since these properties are in the main referable to the existence of the nerve membrane, they do not undergo any qualitative change with variations in the cross section of the external conductor. But in the case of an externally applied current, the fact that in a strict sense nerve is not a linear conductor may, under given conditions, play an important role

Diagram VI of Fig 1 illustrates the spread into the nerve trunk of the current delivered by an external circuit *c*, with the cathode at the fork itself. This spread, be it emphasized, is the spread of current in a volume (cylindrical) conductor. It is not electrotonic spread. In the immediate neighborhood of the fork the current, while spreading into the nerve trunk, crosses the membrane of fibres *T* inwards, and therefore creates in them an anodal zone in which the excitability is decreased. Thus, the remarkable situation arises that the delivery of a conditioning cathodal shock through circuit *c* must decrease the excitability of fibres *T*, and consequently produce a decrease in the height of the response to testing shocks delivered through circuit *t*. The diagram also indicates that the difference of potential created across the membrane of fibres *T* by the applied current causes an electrotonic flow of current in fibres *T* ahead of the fork. Needless to say, diagram VI of Fig 1 is only qualitative, and in depicting the electrotonic spread along fibres *T* ahead of the fork, the diagram is intended merely to explain the fact that with electrodes on nerve *T* ahead of the fork there is recorded through *c* a conditioning shock with a polarity opposite to that obtained with electrodes placed on the nerve trunk

In addition to this fact, the experiment illustrated by the curves in Fig 4 is proof that diagram VI of Fig 1 is correct in its essential features. Curves *I* and *II* (Fig 4) were obtained with the conditioning and the testing circuits having a common electrode at the fork. This electrode in all cases was the cathode of the testing circuit, but it was the cathode of the conditioning circuit for curves *Ia* and *Ila*, and the anode for curves *Ib* and *Ilb*. The conditioning shock was subliminal for curves *Ila* and *Ilb*. Its effect on the tested fibres, i.e. depression of excitability when the shock was cathodal and enhancement when it was anodal, is in agreement with the predictions of

are indicated with fair approximation by the arrows on the records labeled *S* of Fig 5 (cf too, Fig 6, *S*)

In view of these data it is clear that during phase *a* of curves *II* to *IV* the end of the utilization period of the testing pulse coincided with the first phase of the outward membrane current in the conditioning fibres (Fig 1, *IV*, 1) The depression, therefore, is readily explainable in terms of the superposition of the effect of an anodal current on the effect of the testing stimulus Similarly it is found that phase *b* has its crest approximately when the crest of the action potential of the conditioning fibres (Fig 1, *IV*, *Cr*) coincides with the end of the utilization period of the testing pulse of current In the absence of a satisfactory theory of electric stimulation of nerve it is impossible to explain why the time integral of the effect of two successive phases of conditioning currents acting during the utilization period of the testing stimulus was zero, and we must limit ourselves here to a statement of the experimental fact Finally, the *c* phase of curves *II* to *IV* corresponds to the delivery of the testing pulse at such times when its utilization period ended during or after the third phase of the action currents of the conditioning impulses The *c* phase is, therefore, analogous to phase *d*₂ of the standard curve Like this, it long outlasts the detectable flow of the action currents (see later, residual interaction)

Another aspect of the role played by the utilization period of the testing current in determining the shape of the interaction curve is illustrated by the experiment presented in Fig 6 Curves *I* and *II* were obtained under the same conditions as curves *I* and *II* of Fig 5 and do not contain any new fact The interest now lies in curves *III* to *V*, which were obtained after creating a killed end 4 mm beyond the testing cathode

The action potential of the conditioning impulses recorded by pitting the testing cathode against the killed end is reproduced in Fig 6, *III*, *P* If it is taken into account that the record was obtained 4 mm from the killed end of the nerve and that therefore the impulses could scarcely have been conducted past the recording electrode, it becomes clear that the duration of the negative phase of the record corresponds approximately to the duration of the depolarization of the membrane at those points in the immediate proximity of the killed end which still showed the effect of the propagated disturbance (nerve impulse) As long as the membrane of the active fibres was depolarized, the demarcation current of these fibres was decreased, while that of the inactive fibres was increased (14) Consequently the duration of the negative phase of record *P* in Fig 6, *III*, is approximately equal to the duration of the subliminal stimulus to the *T* fibres which resulted from the activity of the *C* fibres Since this duration was of the order of magnitude of the utilization period of the testing pulse of current, the interaction curve (Fig 6, *IV*, *V*) exhibited an actual phase of facilitation

Often when the experiment is performed on the nerves of hibernating

Effect on the temporal course of the testing stimulus on the interaction curve

The results reported in the preceding section leave no doubt that the interaction between neighboring fibres is referable to the effect of the flow of the action currents of the active fibres, because the temporal course of the interaction has proved to be dependent upon those geometrical conditions of the preparation that determine the manner in which the currents flow through the inactive fibres. Hence an attempt will now be made to elaborate the theoretical argument to account for details in the temporal course of the interaction. The first fact to be considered is that the initiation of impulses in the C fibres by the testing stimulus is not an instantaneous event.

Observations made by Blair and Erlanger (6) revealed that brief shocks of near-threshold strength initiate impulses after a shock-spike time, which under the conditions of the present experiments may be as long as 0.4 msec. As will be noted in Fig. 2, this interval of time is about equal to the interval by which the start of the interaction curve precedes the "foot" of the recorded action potential. Thus the fact that a depression of excitability is demonstrable before the action currents begin to flow has a simple explanation. The excitatory process created by the testing shock is prevented from reaching threshold by the action currents of the active fibres that become operative before the end of the shock-spike time. This phenomenon, it seems, is entirely comparable to that observed in the experiments made by Blair and Erlanger (6), in which impulses were prevented from starting by the delivery of an anodal shock near the end of the shock-spike time of a preceding cathodal shock.

The existence of a shock-spike time contributes to the explanation of the finding that the reversal points of the standard interaction curve do not exactly coincide with the reversal points of the membrane current in the conditioning fibres.

When the testing stimulus sets up impulses after a long latency, the interaction curve undergoes important changes. For instance, in the experiment shown in Fig. 5, the shape of the stimulating shock was the only variable. For curve I the stimulus was an ordinary induction shock, while for curves II to IV the stimuli consisted of long pulses of current. In the case of curve II, as shown by the accompanying tracing of an oscillograph record, the pulse of the current was rectangular, but in the case of curves II and III the rate of rise in the current was decreased by introducing a suitable condenser into the circuit.

The curve obtained with induction shocks (Fig. 5, I) presents the ordinary three phases (d_1 , f , d_2) of the standard curve. But at first glance curves II to IV seem to bear no relation to the flow of the action currents of the traveling impulses. Nevertheless, upon careful analysis it will be discovered that they are distorted versions of the standard curve displaced toward and into the region of negative times, and although a phase of facilitation seems to be missing, the curves exhibit phases (a , b , c) that do correspond to the three phases (d_1 , f , d_2) in the standard curve I.

The analysis demands some care but it is not difficult. Zero time in the

tial, is the electric sign of the excitability changes induced in the nerve fibre by subliminal currents, an increase of electric excitability corresponding to a negative slow electrotonus and a decrease to a positive slow electrotonus

Under conditions such as these it was to be expected that the excitability changes which are observed in interaction experiments after the flow of the

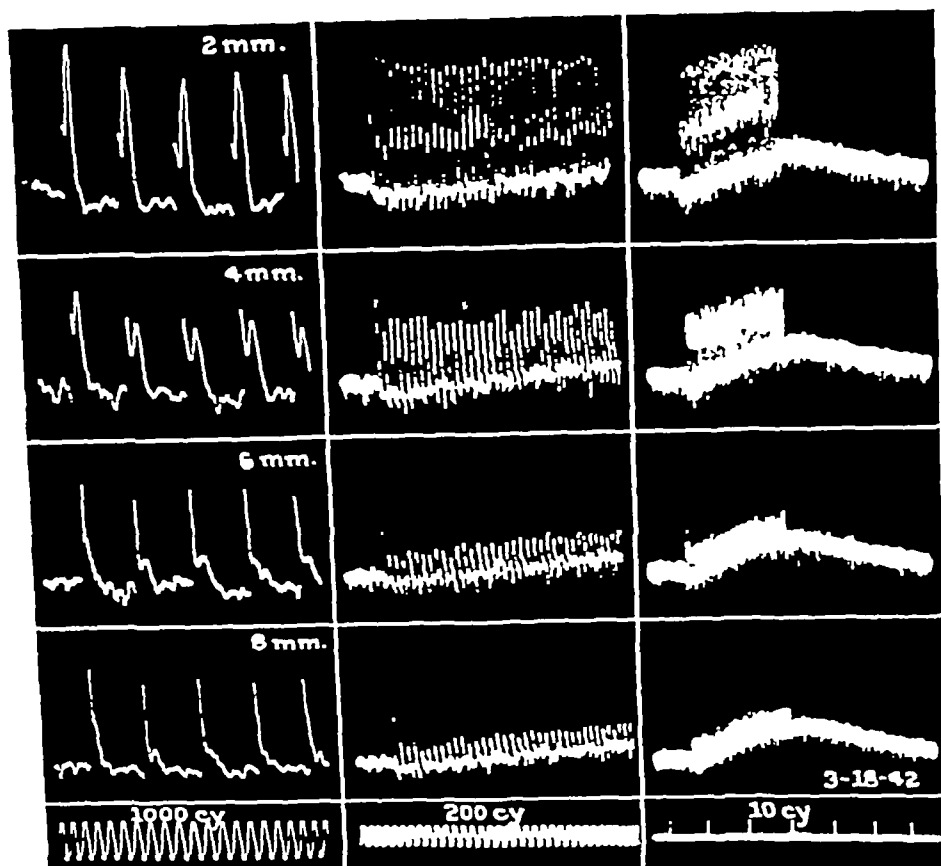


FIG 7 Changes in the membrane potential of the tested fibres induced by impulses traveling along the conditioning fibres Stimulating electrodes on the medial, recording on the lateral peroneal nerve (cf Fig 2, II) The distances indicated on the records are distances of the first recording electrode from the fork, *i e*, they are the distances through which the potential changes had been electrotonically transmitted from the trunk of the nerve, where the changes had been produced, to the point at which they were recorded The amplification was increased as the distance was increased, but the different amplifications were not noted

conditioning action currents has subsided (residual interaction), would be accompanied by the existence across the membrane of a slow electrotonic potential produced by the preceding flow of action currents Experiments like that illustrated by the records in Fig 7 prove that the expectation was correct

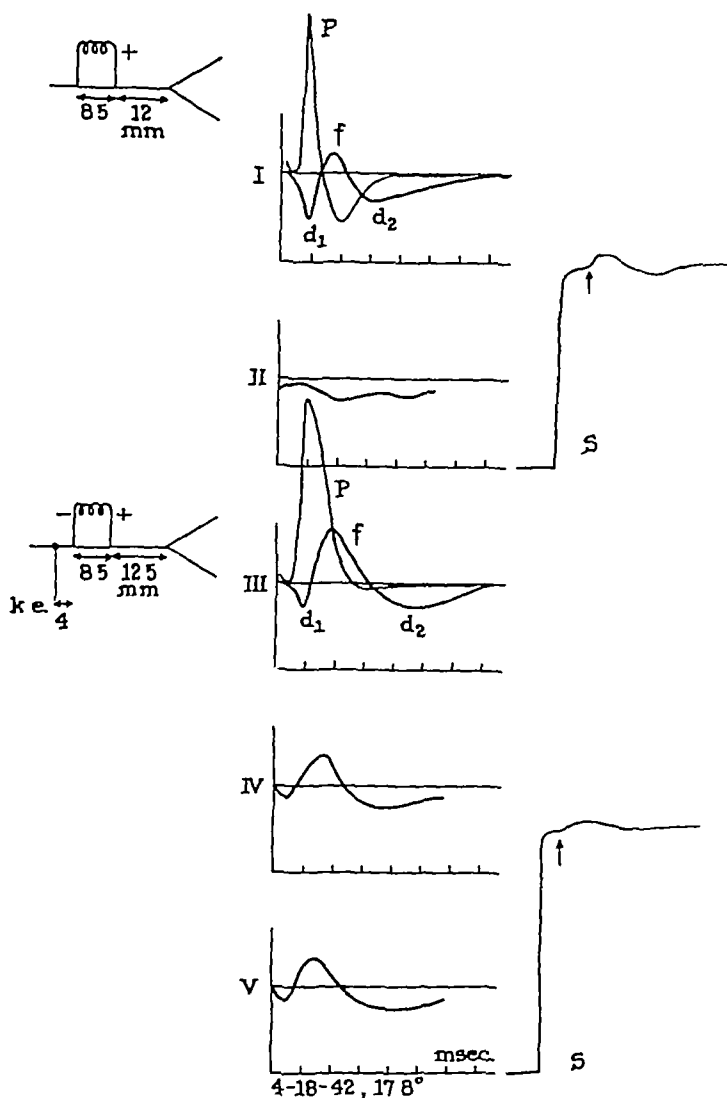


FIG 6 Modifications of the interaction curve resulting from the proximity of the testing cathode to the killed end of the nerve. Curves *III* and *IV* were obtained immediately after creating the killed end, *V*, half an hour later. Induction shocks were used for curves *I* and *III*, rectangular pulses of current for curves *II*, *IV* and *V*. Tracing *P* upon curve *I* was made from a record of the difference of potential established between testing anode and cathode during the passage of the conditioning volley of impulses. Tracing *P* upon curve *III* was made from a record of the difference of potential established by the conditioning volley between the testing cathode and the killed end.

interaction it may be postulated that in the central nervous system the action currents of active elements will produce an effect upon neighboring elements, through which they flow, in exactly the same manner as if the current had been produced by an external circuit or by potential gradients in the tissue, such as those recently described by Libet and Gerard (21). But the problem of ascertaining the effect of the activity of a given set of elements (nerve fibres or somas of neurons) upon neighboring elements must be analyzed in each individual instance, taking into account the anatomical relationship of active and inactive elements, as well as the temporal course of the interacting stimuli. It may be expected that under certain conditions interaction mediated by the flow of action currents will result in increasing the effectiveness of a given stimulus (facilitation), while under other conditions the interaction may result in decreasing the effectiveness of the stimulus (inhibition).

In this connection it is of interest to note that the interaction of neighboring elements may be the explanation of the early inhibition of spinal motoneurons recently described by Renshaw (28) and Lloyd (22), because the superposition of the action currents of neighboring synaptic endings or of somas and synaptic endings, may result in a reduction of the effectiveness of the testing synaptic stimulus (inhibition) in a manner similar to that observed during the first phase (depression) of the standard interaction curve (Fig. 2).

If this assumption were correct, there would still be no need of postulating for the central nervous system the existence of elementary mechanisms not known to exist in peripheral nerve (cf. 11, 12, 23, 26).

Insufficiency of the theoretical argument In the discussion of the interaction curve at the level of the fork, emphasis has been placed upon the fact that the theoretical argument underlying the discussion is a purely qualitative one. This fact cannot be overemphasized.

The effect of the action currents of fibres *C* upon the tested fibres *T* has been evaluated by assuming that the flow of the action currents of *C* is independent of the presence of *T*, while in fact the flow of the action currents of a fibre depends upon the constitution of its external conductor. It is precisely for this reason that the interaction of neighboring fibres results in a change in the speed of conduction of impulses (17). However, a theoretical analysis of the interaction based upon a mathematical study of the flow of action currents would be an exceedingly difficult task. A valuable recent presentation by Wenberg (32) of the theory of the steady state in the cylindrical core conductor gives the reader a clear concept of the mathematical difficulties that are encountered in an analysis of the distribution of currents in nerve, when the usual linear conductor approximation is abandoned. In regard to the interaction problem itself, Lord Kelvin's (19) treatment of an analogous problem of the theory of the submarine cable illustrates the complexity of the situation. To increase the difficulties there is the fact that a satisfactory theory of the electric stimulation of nerve is not available.

frogs, the arrival of a C volley at a freshly killed end results in the initiation of impulses in the T fibres (Hering's phenomenon, cf literature in the Introduction) An important detail to be observed in curves III to V of Fig 6 is that the interaction curve did not fail to exhibit its third phase (depression) after the production of the killed end

RESIDUAL INTERACTION

It will be observed in all the interaction curves of Fig 2 to 6 that the third phase of the excitability change long outlasts the detectable flow of the action currents The curves in Fig 2 to 6, however, do not extend throughout the whole period of residual changes of excitability In agreement with the results obtained by Otani (27), it has been found in the present research that the phase of depression may last for as long as 20 to 30 msec or even longer, and that it may be followed by a fourth phase of facilitation which occasionally has been observed to last for intervals of time up to one second The changes in excitability are small and cannot be measured with accuracy either during the late part of the phase of depression or the following phase of facilitation, but the observations that have been made conclusively prove their existence

Since the residual change of excitability is observed while no action current flows through the conditioning fibres, it must be explained in terms of changes produced in the testing fibres by the previous flow during the early phases of the interaction

Arvanitaki (3) reports that when the conditioning action currents are subliminal for the tested fibre, they induce in the latter a change similar to that induced by a subliminal electric shock which is delivered through an external circuit An analogous phenomenon should be expected to occur and in fact, as will presently be shown, it does occur in frog nerve Unfortunately, a detailed comparison of Arvanitaki's observations with the results reported in this paper is not possible, because a correspondence between the so-called subliminal or uncondacted response of invertebrate nerve and subliminal processes in vertebrate nerve has not as yet been established in a satisfactory manner (cf 5, 16)

Observations made by Lorente de N6 and Davis (25) have revealed that the electrotonic potential of frog nerve, that is to say the increment of the membrane potential which is produced by an externally applied current, has two components, which, in view of the different rates of their establishment and decay, may be called the "fast" and the "slow" electrotonic potential, or simply "electrotonus" The two components of the electrotonus are produced by cathodal as well as by anodal currents, and as well by currents above or near the rheobase as by currents below the rheobase, in fact, the two components are produced by any current, however small, Without entering into details* it may in general be said that the slow component of the electrotonus, which as a rule is only a fraction of the total electrotonic poten-

* This problem is discussed in detail by Lorente de N6 and Davis in their forthcoming monograph

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Records obtained with the electrodes placed on the trunk of the sciatic nerve do not yield information which is sufficient to decide whether the recorded potentials are referable to the conditioning or to the tested fibres. But no doubt can arise when the records are obtained from the tested fibres alone, as is the case when the recording electrodes are placed on the branch of tested fibres ahead of the fork (cf diagram in Fig 3, II). In the experiment shown in Fig 7 the second recording electrode was maintained on the killed end, while the first recording electrode was placed at the distance from the fork (2, 4, 6 and 8 mm) indicated on the records. These records therefore measure changes in the membrane potential of the tested fibres, which had been electrotonically transmitted through segments of nerve of various lengths.

The records in the first column (Fig 7) obtained at a relatively high sweep speed following a sharp deflection, which mainly is an instrumental (capacitative) shock artefact, present a spike-like potential followed by a longer-lasting positive phase that corresponds to the phase of depression in the interaction curve (cf Fig 3, II). The positive phase is more readily seen when the conditioning fibres are made to conduct a train of impulses (cf the middle column of records in Fig 7). Whether elicited by a single conditioning volley or by a train of volleys, the positive potential ultimately is followed by a negative potential that corresponds to the late phase of increased excitability. With single volleys the amplitude of the late negative phase of the electrotonic potential is so small that the deflection may easily pass unnoticed, but with tetanic conditioning the late negative phase is a prominent feature of the records (cf last column of records in Fig 7), a remarkable detail being that the change in the sign of the electrotonic potential takes place while the conditioning tetanus is still going on.

No attempt will be made here to analyze in detail the records of Fig 7. Only the fact will be mentioned that the spike-like potentials observed during the immediate interaction are transmitted along the nerve with greater electrotonic decrement than the slower potential changes that accompany the residual interaction (for an analogous situation cf 24).

COMMENT

One of the reasons justifying a systematic investigation of the interaction of neighboring fibres in peripheral excised nerve is that similar phenomena must be expected to play a role in the central nervous system. As a matter of fact, the observations made by Renshaw and Therman (29) demonstrated the existence in the spinal cord of interaction similar to that taking place in peripheral nerve.

However, observations made on excised peripheral nerve cannot be used directly to interpret happenings in the central nervous system, because the geometrical conditions that determine the flow of the action currents, as well as the temporal course of the testing stimuli, are different in the two instances. On the basis of the information which is available on peripheral

THE ELECTRICAL ACTIVITY OF REGENERATING NERVES IN THE CAT*

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INTRODUCTION

THE EXTENSIVE WORK of Cajal (8), Huber (6) and Young (11) has advanced our understanding of the processes of nerve regeneration significantly and these authors have provided an adequate review of the field. The experiments which are reported here add new data through a study of the electrical properties of regenerating fibers in the tibial, peroneal, and saphenous nerves of the cat and permit correlations between these electrophysiological data and histological information obtained from the same nerves.

It is now well established that regenerating nerve fibers begin as small outgrowths which gradually increase in diameter (4). Therefore, it might be expected that the outgrowths of the regenerating fibers conduct impulses slowly at first and then at increasingly higher velocities as the time of regeneration continues. This expectation is based on the fact that the conduction velocity of mammalian nerve fibers is directly proportional to the diameter as shown by Hursh (7) and Gasser and Grundfest (1). Our data confirm this expectation and describe the rate at which the conduction velocity increases on its return toward normal. Also, since both the velocities and the diameters of the regenerating fibers were measured, the linear relationship between them has been confirmed under new experimental conditions.

Combined electrical and histological analyses of the regenerating nerves was the main objective of these experiments, but other less detailed information on the functional repair of nerves was also gathered by observation and stimulation of the animals used in the course of the experiments.

METHODS

A series of 58 cats were operated upon with aseptic precautions under nembutal anaesthesia. In all of this series, the right sciatic or its tibial and peroneal branches were divided with a scalpel and sutured immediately. Two epineural sutures of fine black silk thread were placed in the sciatic nerve or its branches. The levels of the sutures were varied on the sciatic between the notch and up to 2 cm. distal to the division into the peroneal and tibial branches, and frequently, the peroneal and tibial were sutured separately. In 36 of the animals, the right saphenous nerve was similarly treated. The saphenous nerves also were sutured at various levels between their origin from the femoral and the level of the knee. One or two sutures which frequently included part of the substance of the nerve were placed in the saphenous. During the course of regeneration, observations were made on the ability of the animals to walk with the use of the gastrocnemius and toes. Responses to touch, pin prick, pinching and the placing reaction were also observed.

* This work was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the Cornell University Medical College.

Under conditions such as these the theoretical treatment of the interaction problem can scarcely go beyond a qualitative analysis of the type that has been employed in this paper

SUMMARY

A systematic analysis has been carried out of the changes occurring in the excitability of nerve fibres, induced by impulses traveling in neighboring nerve fibres. The experimental results have been expressed in the form of interaction curves (Fig 2 to 6)

Flow of the action currents of the active fibres through the membrane of the inactive ones is the mechanism underlying the interaction

Two parts may be differentiated in the interaction curve, because besides an immediate interaction, there is a residual interaction which outlasts the flow of the action currents. The residual change in excitability has an electric sign that consists of a change in the membrane potential (Fig 7)

The shape of the interaction curve depends upon the geometrical conditions determining the flow of the action currents through the inactive fibres and upon the temporal course of the testing stimulus

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When the nerves had regenerated for a desired interval, up to 466 days, the cats were decerebrated and used in terminal experiments. The sutured nerves were exposed and stimulated *in situ* with 60 cycle stimuli of variable intensity to observe sensory effects such as increased respiration and reflexes on stimulating afferent nerve fibers and direct motor effects from the stimulation of efferent fibers. The exposed nerves were then excised and used for the electrical and histological studies.

The excised nerves were placed in a moist chamber which was kept at 38°C and was large enough to accommodate 15 cm of nerve length. The electrodes were adjusted from outside the chamber without changing the environment of the nerves. The activity of the nerves in response to electrical stimulation was recorded oscillographically with the methods which are standard in the Laboratories of the Rockefeller Institute. After the electrical information had been secured, portions of the nerves were fixed in chloral hydrate solution and stained with protargol, other portions were fixed in osmic acid.

RESULTS

Electrical responses of regenerating fibers distal to the suture The earliest electrical signs of the activity of the regenerating fibers were detected in the peripheral stump of a tibial nerve 17 days after the division and suture of that nerve. The action potential was very small and was conducted at 0.9 mps, a velocity similar to that of C fibers in normal nerve. A similar potential from the peroneal nerve of a cat 36 days after operation is illustrated in Fig. 1a. This small spike was less than 10 μ V in magnitude and was conducted at a maximum velocity of 0.8 mps between electrodes which were placed at 1.5 and 3 cm distal to the suture. The regenerating fibers of the tibial nerve of this same animal showed more recovery with a conduction velocity of about 18 mps with greater spike height (Fig. 1b). In this experiment, the electrodes were placed 1.5 and 3 cm from the suture, but the velocity was within the same range (*i.e.* 14 mps) when the electrodes were moved 2 cm more distally.

The spikes became larger in nerves which had regenerated longer and showed a greater maximum conduction velocity. The spike from the peripheral stump of a tibial nerve 106 days after operation (Fig. 1c) was conducted at 45 mps and was divided into two conspicuous components, a large potential from a group of rapidly conducting fibers followed by a smaller, longer lasting potential from more slowly conducting fibers. A much later potential within the range of C fibers was always present in these nerves, but is not shown with the rapid sweep speeds used in Fig. 1c or 1d. Figure 1d was taken from the peroneal nerve of a cat 466 days after operation in which the spike was large and conducted at a velocity of 75 mps.

Thresholds for electrical stimulation of regenerating fibers The strengths of electrical stimuli required to elicit activity of the nerve depended upon the placement of the stimulating electrodes. When the response was initiated by stimulating the intact fibers proximal to the suture, weak stimuli within the general range which is normal for large A fibers were sufficient. On the other hand, relatively strong stimuli sufficient to excite normal C fibers had to be applied to the fibers which were growing into the peripheral stump in order to produce activity. As the time of regeneration increased, the stimulus

distances are plotted in the graph of Fig 2 The slope of the line connecting the points is equivalent to the conduction velocity of the most rapidly conducting fibers, this statement rests on the assumption that the records represent the same fibers at each point The possibility that the fiber composition which produced the potential remains constant is likely with this electrode arrangement since only those regenerated fibers which reached the distal, stimulating electrodes without being lost in side branches or in other

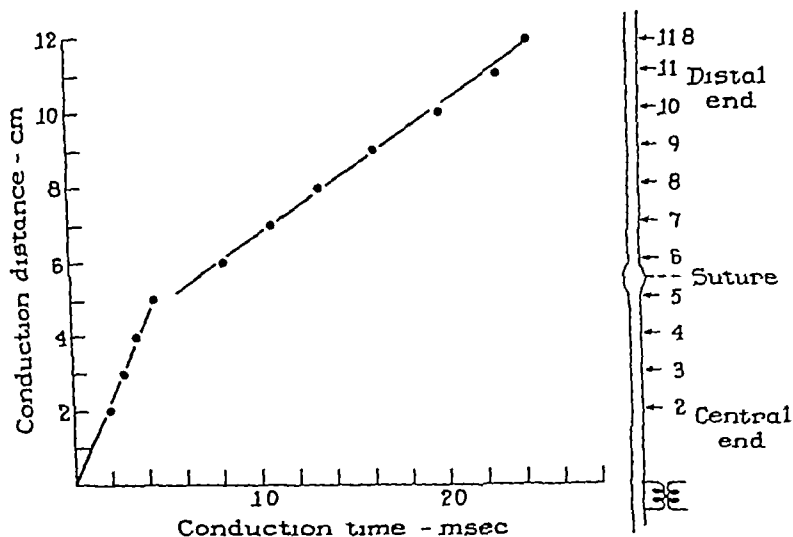


FIG 3 The conduction velocities of regenerating fibers central and distal to the suture from a tibial nerve at 90 post-operative days The stimulating electrodes (central) were stationary while the recording electrodes (arrows) were moved Conduction velocities are 110 mps centrally and 34 mps distally

obstructions are stimulated, and all of the fibers of this caliber must originate from the proximal stump The continuity of the line also indicates that the same fibers were involved throughout this series of measurements

The abrupt change in the conduction velocity at the suture (Fig 2) is striking The velocities at shorter conduction distances represent conduction entirely within the regenerating fibers of the peripheral stump, and in the experiment illustrated (saphenous nerve at 58 post-operative days), the slope in this part of the graph shows a velocity of 23 mps The portion of the curve plotted at longer conduction distances represents conduction continued proximal to the suture, and here the conduction velocity jumps suddenly to 50 mps These data show clearly that the more slowly conducting fibers of the distal stump must have been outgrowths of faster fibers from the proximal stump However, it is interesting to note that in this experiment, the central conduction velocity of 50 mps is considerably slower than that of normal saphenous nerve and slower than the most rapid fibers which are present in this central portion of the regenerating nerve In several other experiments of

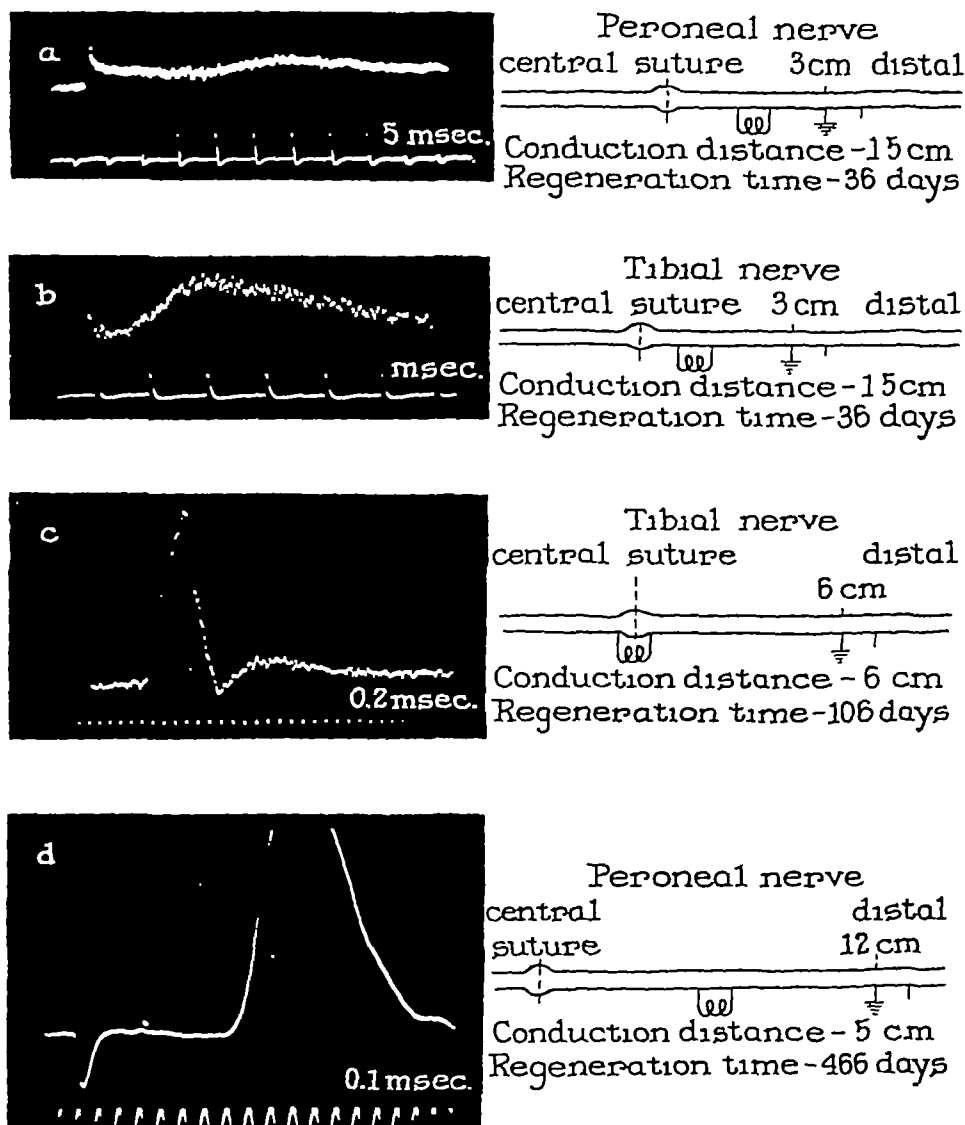


FIG 1 Action potentials recorded from the peripheral stumps of regenerating nerve fibers. The diagram adjacent to each record explains the arrangement of the recording and stimulating electrodes. *a* and *b* are from the peroneal and tibial nerves of the same cat 36 days after suture. *c* is from the tibial nerve at 106 days, and *d* is from the peroneal nerve of a cat at 466 post-operative days. The amplification was progressively reduced from *a* through *d*.

tant in connection with the "safety factor" of the nerve impulse (5, 10) when more information is available concerning the nature and magnitudes of the bioelectric potentials produced by nerve fibers. A condition similar to the one here described probably also exists normally in the central nervous system, where large fibers terminate in the fine fibers of the neuropile

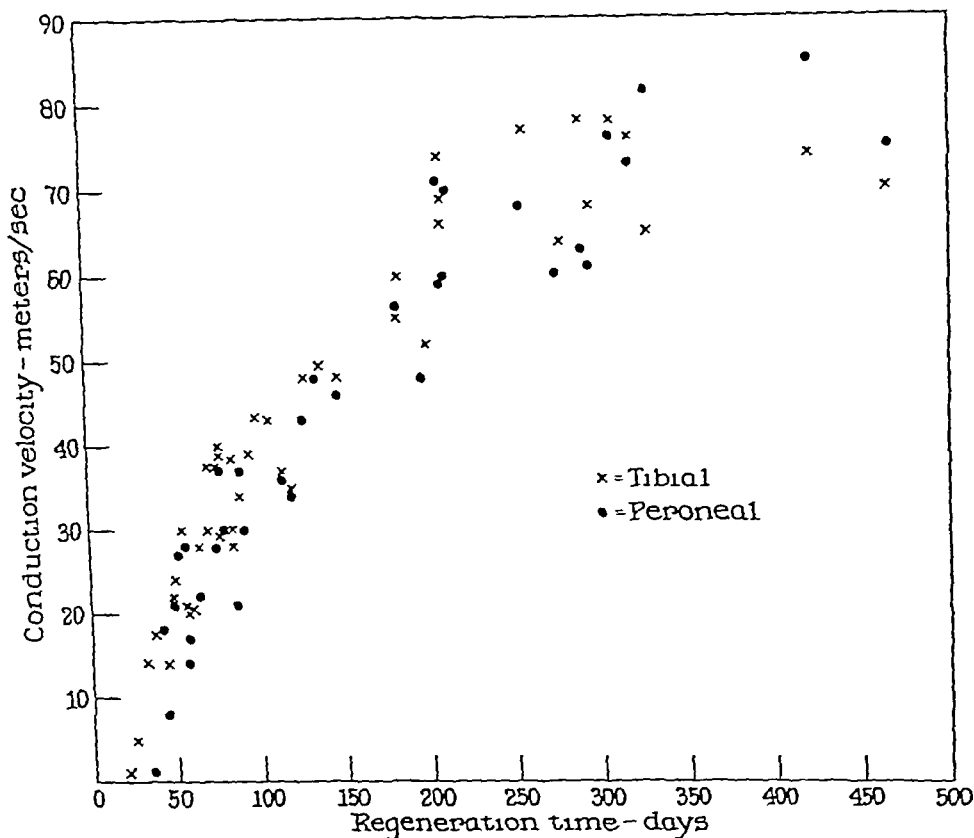


FIG 4a. Graph of the relationship of the maximum conduction velocity of fibers in the peripheral stumps of the tibial (crosses) and peroneal (circles) nerves to the time of regeneration following suture. Each velocity plotted was determined from a different nerve by the graphic methods of Fig 2 and 3

Change in the maximum conduction velocity with regeneration time The conduction velocities of the most rapidly conducting fibers which were present in the regenerating peripheral stumps of the tibial, peroneal and saphenous nerves increased with the time of regeneration as was indicated by the relatively crude data discussed with Fig 1. With the use of series of records from each nerve and with the more accurate graphic method of determining velocity, the conduction velocities were determined for the entire series of nerves. Each point on the graph on Fig 4a represents data from a different

strengths which were required to excite the peripheral stump steadily decreased. These facts indicate that the electrical properties of the regenerating fibers of the peripheral stump were different from those of their "parent fibers" in the proximal stump, and that the transition took place in the region of the suture.

The thresholds described above were obtained with single shocks of very brief duration, but on a few nerves, alternating currents of 60 cycles per

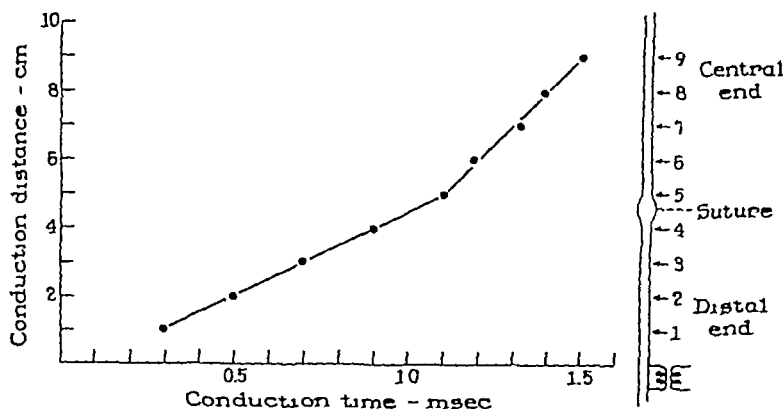


FIG. 2 The maximum conduction velocities of regenerating fibers distal and central to the suture obtained by plotting the conduction distance against the conduction time of the action potential. The stimulating electrodes were stationary while the recording electrodes (arrows) were moved. Velocities are 23 mps in the distal stump and 50 mps in the central stump. Saphenous nerve at 58 post-operative days.

second and repetitive shocks of short duration also produced repetitive responses. No detailed analysis of refractory periods was attempted.

The conduction velocity of regenerating fibers. Of the various properties of nerve which can be determined electrically, the conduction velocity is perhaps the best suited for quantitative study. The velocities were determined along the lengths of the nerves and across the suture lines of the regenerating saphenous, tibial, and peroneal nerve. Distinct differences were found in the conduction velocities of the proximal and distal portions with respect to the suture.

The method of determination of the maximum velocities of conduction consisted of analysing a series of action potential records taken at several conduction distances along a nerve. Although many of the possible electrode arrangements were used in these experiments, two of the most useful are illustrated in Fig. 2 and 3. In the first arrangement (Fig. 2), the electrodes were moved stepwise to provide greater and greater conduction distances. In the experiment of Fig. 2, the stimulating electrodes were kept at a point 4.6 cm distal to the suture while the pair of recording electrodes were moved to a variety of points both distal and proximal to the suture. The conduction times of the most rapidly conducted spikes at each of the various conduction

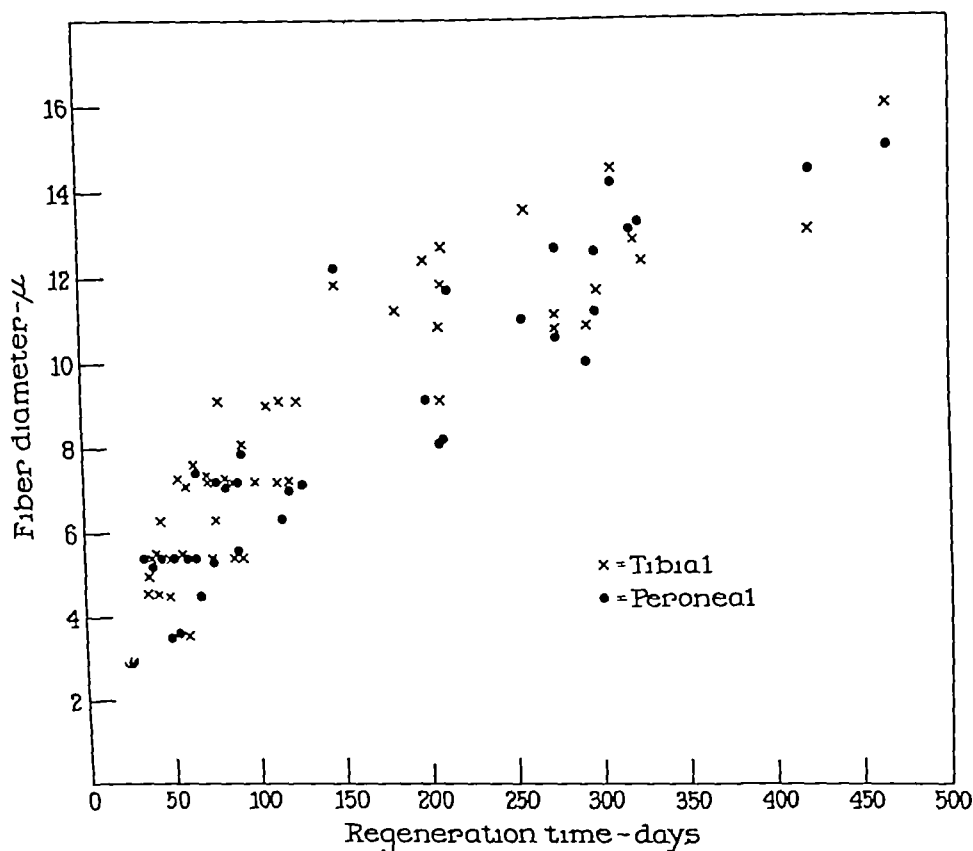


FIG 5a Graph of the outside diameters of the largest fibers of the distal stumps of the regenerating tibial (crosses) and peroneal (circles) nerves plotted against the time following suture

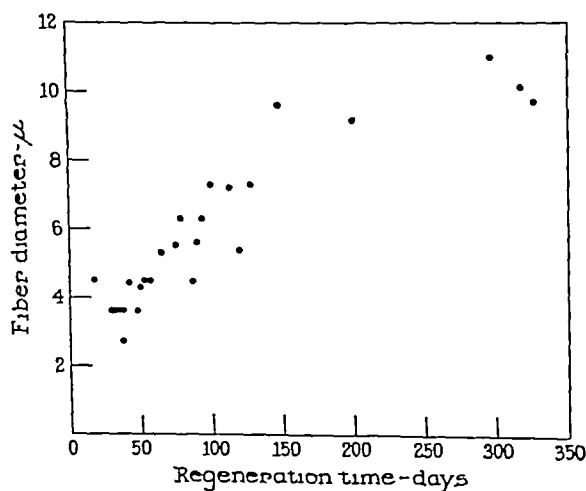


FIG 5b Same as Fig 5a, but for the saphenous nerve

this type, the conduction of the regenerating fibers central to the suture was relatively low. Examples of this were found with the saphenous, peroneal and tibial nerves without definite relationship to the time of regeneration. In most instances, however, the central conduction velocity was in the range of the maximum normal values. From these results, it seems that the most rapidly conducting fibers of the peripheral stump are not necessarily outgrowths from the most rapidly conducting fibers of the proximal stump.

In the second electrode arrangement which is essentially the reverse of that shown in Fig 2, the proximal pair of electrodes were held stationary while the distal pair were moved to various conduction distances. In the graph of Fig 3 from the tibial nerve of cat at 90 post-operative days, the measurements of conduction times at the shorter conduction distances represent conduction exclusively within the proximal stump. At the longer distances, the fibers are followed into the peripheral stump. The velocity of the central conduction obtained from the slope of the graph of Fig 3 is 110 mps, a figure within the normal range of A fibers in the tibial nerve. However, in this experiment, there was no assurance that the fibers which conducted at this rapid rate were exactly the same fibers as those whose response was recorded from in the peripheral stump. The discontinuity of the curve in the region of the suture indicates that the identical fibers were not always traced into the distal stump. Although this discontinuity was not striking in Fig 3, several experiments showed even greater separation of the two slopes.

The slope of the line of Fig 3 at the longer conduction distances including the distal stump shows a conduction velocity of 34 mps. Although the slope and, therefore, the velocity was constant for at least 6 cm, a situation which was frequently repeated in other experiments particularly at longer post-operative intervals, occasionally the velocity decreased as the electrodes were moved distally from the suture. In one such experiment in a tibial nerve at 106 days, the velocity changed from 58 mps at 3 cm distal to the suture to 49 mps at 5 cm and to 36 mps at 6 cm from the suture. This decrease was gradual in many cases, but sometimes it was abrupt as if a branch of more rapidly conducting fibers had been given off or lost. With the first electrode arrangement (Fig 2), a gradual decrease in velocity was not observed indicating that the recovery of conduction velocity occurred at an even rate for the whole, or a considerable length of the fibers in the distal stump. Since these results are conflicting, no definite conclusions can be reached to substantiate or disprove the fact that fibers acquire their myelin sheaths and diameters beginning at the proximal end (6) or that the myelin progresses down the regenerating nerve as a definite myelin front (11).

We do not know of any earlier, direct evidence that an action which is propagated at high velocity in normal nerve fibers is also capable of propagating into slowly conducting extensions of the fibers, or that the impulses which originate in thin, slowly conducting fibers are also capable of propagating into large, rapidly conducting fibers. This evidence should be impor-

have already given detailed information on the rate at which fibers grow out into the peripheral stump. We have not, therefore, duplicated their systematic experiments but we have in a number of experiments assured ourselves that the rate of return of functional sign was more than 3 mm per day, a figure which agrees with the rapid recoveries recently noted by Gutmann, *et al* (3)

Change in the maximum diameter with regeneration time The outside diameters of the largest fibers were measured in cross sections of the same ex-

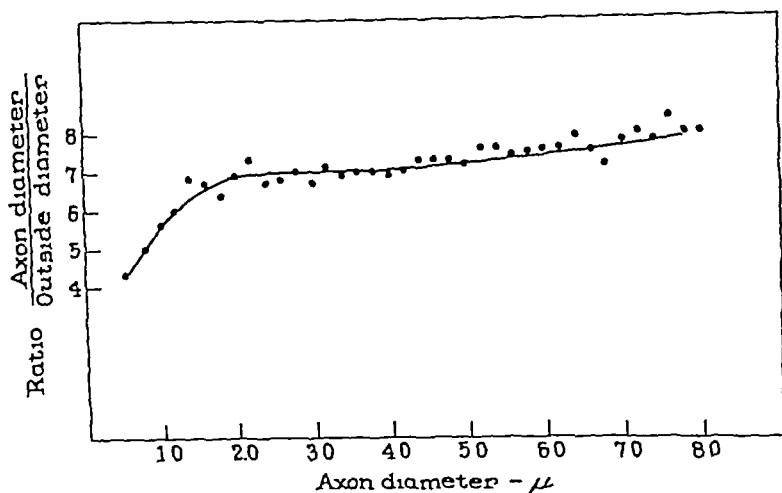


FIG 7 Graph of the axon-diameter outside-diameter ratio plotted against the outside diameter. Taken from the distal stump of right sural nerve after 200 days regeneration time

cised nerves which had been used in the electrical experiments. Although there were errors due to fixation in osmic acid and errors due to the small percentage of large caliber fibers in the regenerating nerves, the diameters were clearly seen to increase with time as proven by Gutmann and Sanders (4). In Fig 5a, the maximum diameters of the regenerating fibers of the peroneal (circles) and tibial (crosses) were plotted against the regeneration times. The results from the saphenous nerves showed a similar curve, but the fibers were consistently smaller (Fig 5b).

The fibers of early regenerating nerves were small, but these were not plotted on the graphs in Fig 5a and b since the debris from the degenerating fibers complicated the osmic preparations. However, the rest of the curves show a gradual increase in diameter with time at a decreasing rate in a manner similar to that of the velocity-time curves of Fig 4a and b. In this series of nerves, the largest diameter was only 16μ at 466 days whereas there are normal fibers larger than 20μ . This incomplete return of fiber diameter does not match the findings of Gutmann and Sanders (4) wherein normal diame-

nerve and shows the maximum conduction velocity for the first 10 cm of regenerated tibial (crosses) and peroneal (circles) nerves, plotted against the time allowed for regeneration of that nerve. The results obtained from the saphenous nerves are shown separately in Fig 4b. Maximum normal velocity in this nerve is lower than in the tibial and peroneal nerves.

Perhaps the most striking feature of Fig 4a and b is the fact that the regenerating fibers "matured" or approached the normal velocity very slowly. In none of the experiments, even with the nerves which had been allowed to regenerate for 466 days was there any complete return to the velocities expected from normal nerves. In fact, after this period of regeneration the conduction velocity was still less than 80 per cent of the normal conduction velocity.

Neuromuscular transmission in regenerating fibers. Direct stimulation of

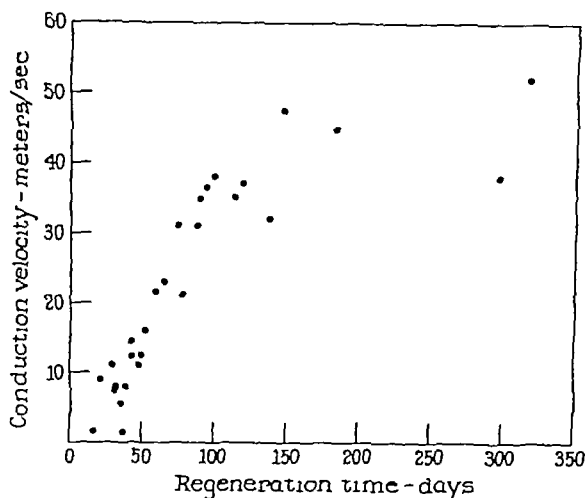


FIG 4b Same as Fig 4a, but for the saphenous nerve

the regenerating nerve fibers produced muscle twitches as early as the 23rd day after suture (gastrocnemius). Observation of the movements of the animals, however, showed that they began to use the gastrocnemius muscle only after about 50 or 60 days, although considerable reinnervation of the muscle had taken place by that time. The inadequate ability of the central nervous system to use reinnervated muscles may be caused by a number of factors. In the first place, section and suture introduces the very considerable hazard that reinnervation (both afferent and efferent) may establish new pathways entirely inappropriate for carrying out the pattern of muscle movements required of the animal. In nearly all the animals of our series (where section and suture were carried out, condition of neurotmesis [9]), the normal use of the operated limb never appeared to be completely regained. In contrast, in a series of animals in which the nerves had been crushed (axonotmesis [9]) we have observed that the operated limb was restored to nearly normal use several months after the operation. In these cases, presumably, the majority of the regenerating fibers grew back into their original connections and the normal pathways were therefore restored. These observations confirm Gutmann (2). The first situation (severance of the fibers) unfortunately, is the condition most common in war and industrial injuries. On the basis of our experimental data, the return of normal function after nerve section is, therefore, beset with this difficulty.

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The slowed conduction velocity of the regenerating nerve fibers is probably another factor which is also operative in the inability of animals to use their operated limbs normally, since the timing and pattern of both afferent

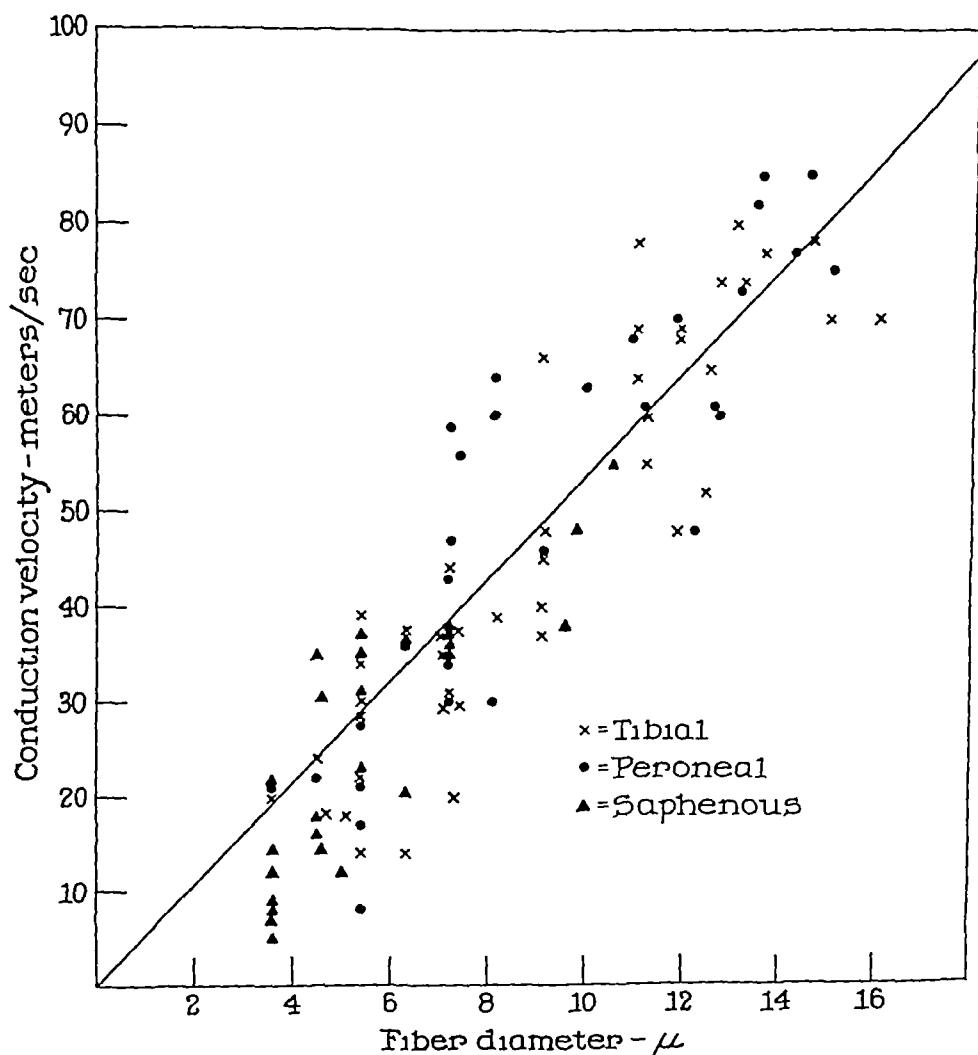


FIG 6 Graph showing the approximately linear relationship between the maximum conduction velocities and the outside diameters of the regenerating fibers of the tibial (crosses), peroneal (circles) and saphenous (triangles) nerves

and efferent impulses must be considerably disturbed because of the slowed conduction velocity

Rate of growth of regenerating nerve fibers Young and his collaborators

EFFECT OF TEMPERATURE ON THE OXYGEN CONSUMPTION OF BRAIN TISSUE*

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THE EXTENSIVE literature on the metabolism of excised mammalian brain (for reviews cf., 10, 30) is concerned for the most part with data obtained at about the mean body temperature of the animal studied. However, in this laboratory the effect of variation in environmental temperature on the action of chemical agents which augment or depress tissue metabolism has been studied for several years (15, 16). In the control experiments accompanying this work the oxygen consumption of cerebral cortex slices from 345 adult albino rats has been measured at temperatures ranging from 0.2° to 47.5°C. Most of the observations were made at or below 37.5°C, but for 35 animals measurements were made at supranormal temperature levels. This large body of fundamental data, certain derivative statistics and observations on the reversibility of the effects of high temperatures on the oxygen uptake of excised rat brain are presented in this paper.

METHODS

The methods used in the preparation of tissue slices from the cerebral cortex of the rat and in the measurement of oxygen consumption have been described previously in considerable detail (16), therefore only a brief summary of method is given here. Suitable slices of cerebral cortex were obtained using a razor blade and lucite template in a moist box. Oxygen uptake was measured by the Warburg method. The suspension medium (liquid phase) in the respirometer vessels was Ringer's solution buffered at pH 7.35 with sodium phosphate mixtures in a final concentration of M/100. This medium contained 0.2 per cent glucose. The gas phase was oxygen. Four constant temperature baths were available, two of which were equipped with cooling units. Thus it was possible to make concomitant runs on brain slices from a given animal at four temperature levels, two of which could be below room temperature. More uniform data can be obtained in this way than in successive runs on different animals. All temperatures were constant to $\pm 0.02^\circ\text{C}$ or better. Fifteen minutes of thermo-equilibration preceded each run. Oxygen consumption was measured in c mm, N P T, per mg dry weight per hour (Q_{O_2}). Determinations on the same brain at a given temperature were made either in duplicate or in triplicate.

RESULTS AND DISCUSSION

Under the conditions of these experiments the oxygen consumption of cerebral cortex slices remained constant for several hours at all water bath temperatures from 0.2° to 37.5°C. At 40°C the rate of oxygen uptake was constant for 2 hours or more in about half the runs, in the others it began to decrease as early as thirty minutes from the time the respirometers were set. Possibly changes in environmental conditions might raise the temperature at which a constant rate could be obtained (4). However, in dealing with the

* Supported by grants from the John and Mary R. Markle Foundation and from the Fluid Research Fund of the Stanford University School of Medicine.

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ters were obtained after 300 days. However, they employed rabbits and crushed the nerves while the nerves of this series of cats were sutured.

The relationship between the conduction velocity and the fiber diameter Since the maximum velocities of the fibers and the maximum diameters of the same regenerating nerves were measured, the two functions can be plotted against each other as in Fig. 6. In this graph, the data from the tibial (crosses), from the peroneal (circles) and from the saphenous nerves (triangles) are plotted together. The points scattered about a straight line. Therefore, these results show the same linear relationship between fiber diameters and conduction velocities as found by Hursh (7) and Gasser and Grundfest (1).

The ratio between axon diameter and outside diameter of regenerating nerve fibers In our data on regenerating fibers, as in the data of Gasser and Grundfest (1), there is a deviation from the linear relationship for the small fibers. Gasser and Grundfest showed that this deviation may be due in normal fibers to the fact that the ratio between axon diameter and outside diameter falls considerably for the small fibers. We have measured the axon diameter and outside diameter of 1569 fibers of a sural nerve which had regenerated for 200 days. The curve obtained for the ratio between these diameters (Fig. 7) is similar to that obtained by Gasser and Grundfest.

SUMMARY

1 Action potentials and microscopic preparations of regenerating tibial, peroneal and saphenous nerves of cats were studied at intervals up to 466 days following primary suture.

2 Action potentials were detected as early as 17 post-operative days and were small and slowly conducted at velocities less than 1 mps. At longer post-operative intervals, the potentials increased in size, velocity, and complexity showing at least two prominent components plus a C wave.

3 The thresholds for electrical stimulation of regenerating fibers were high peripherally and low centrally to the suture. At longer post-operative intervals, the thresholds of the distal fibers became progressively lower.

4 The maximum conduction velocities were measured for regenerating fibers on both sides of the suture, and abrupt changes in velocity were noted as the impulse passed the suture. Also, the most rapidly conducting fibers of the distal stump were not necessarily projections from the most rapidly conducting fibers of the central stump.

5 The progress of the return of conduction velocity in the fibers distal to the suture was slow and the velocities increased at a constantly decreasing rate with respect to time. The return of maximum fiber diameter followed a similar course.

6 The relationship between the maximum conduction velocity and the maximum fiber diameter in the regenerating nerve was approximately linear.

When the data were subjected to the conventional mathematical procedures for determining temperature coefficients (4) it was found that over the range 0.2° to 37.5°C $\log Q_{O_2}$ was not a linear function of either the Centigrade temperature, the reciprocal of the absolute temperature or of log temperature. This is usually the case when biological processes are studied over a wide temperature range (4, 7, 29, 33). However, the graph of $\log Q_{O_2}$ as a function of Centigrade temperature was approximately linear over the range 10° to 37.5°C . This is made evident in Fig. 2.

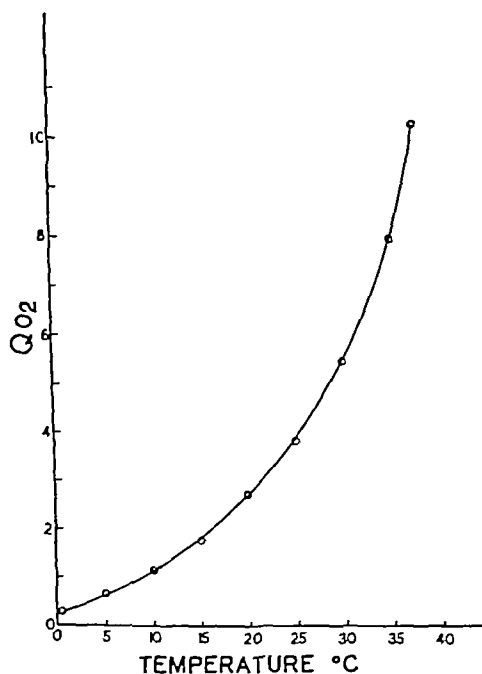


FIG. 1 Graph showing Q_{O_2} as a function of temperature

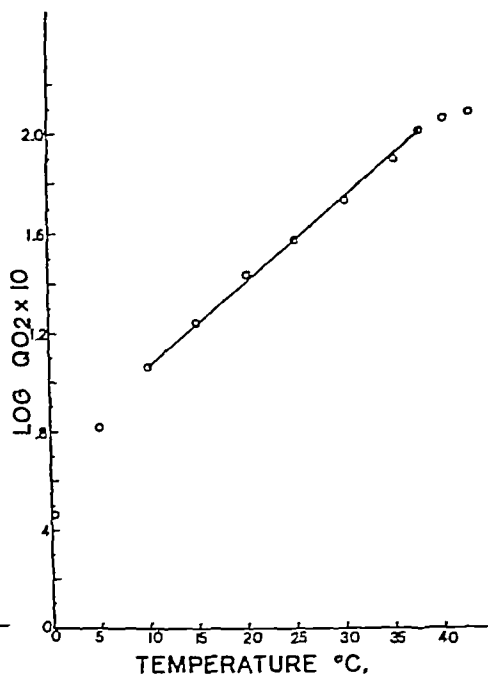


FIG. 2 Graph showing $\log Q_{O_2}$ as a function of temperature in degrees Centigrade

The curve in Fig. 2 was fitted to the experimental points by the method of least squares. The equation for this curve is

$$\log Q_{O_2} = -0.2438 + 0.0328t$$

where "t" represents Centigrade temperature. The value of the van't Hoff coefficient, Q_{10} , is given by the slope of the curve in Fig. 2 (over a 10° interval) and is antilog 0.328 or 2.128. This differs but slightly from the value of 2.102 previously reported from this laboratory for a smaller number of animals (19).

No data were found on which to base a comparison between the influence of temperature on the oxygen consumption of the intact rat, uncomplicated by cold defense reactions, with the relations shown in Fig. 1. In the absence

The curves of Fig 3 for 45° and 47.5°C exemplify Blackman's three "laws" (5) describing the influence of supraoptimum temperatures on metabolic processes, *viz* that at such temperatures metabolism shows a progressive decrease with time, that this decrease is faster the higher the temperature, and that the decrease is most rapid initially

Beside these features, the 45° and 47.5° isotherms in Fig 3 converge toward a final common value of Q_{O_2} , which may be termed the thermostable Q_{O_2} . It is shown in Fig 4 that the same change is occurring at 42.5°, although more slowly. The thermostable Q_{O_2} amounts to about 10 per cent of the Q_{O_2} of fresh cerebral cortex slices at 37.5°, and is approximately equal to the fraction of oxygen uptake which is stable toward various chemical inhibitors of oxygen consumption in excised tissue (16, 17). We found that the thermostable Q_{O_2} was not affected by either 0.024 M propazone or 0.1 M sodium azide. Both compounds, in these concentrations, reduced the oxygen consumption of cerebral cortex slices (at 37.5°C) to the inhibitor stable level. Thus there is considerable qualitative and quantitative similarity between the thermostable, inhibitor stable and relatively cold stable (cf 15) fractions of rat brain respiration. It is tentatively suggested that these fractions represent the same group of biological oxidation processes.

It is evident from inspection of the curves of Fig 1 and 3 that, insofar as Q_{O_2} is concerned, Dixon (11) is mistaken in stating that in excised brain, "a rise in temperature from 37° to 42° only produces a very slight increase in metabolic rate, whilst on raising the temperature to 45°C the metabolism increases enormously." This view is not in harmony with either the observations of Himwich and associates (21) on brain brei, of Brachet and Bremer (6) on minced frog brain or our findings on rat cerebral cortex. Furthermore, in our opinion, the data published by Dixon are too few and too variable to serve as the basis for generalization.

The type of heat effect illustrated in Fig 3 has been demonstrated in many and diverse biological processes and forms (4, 7, 22). It is therefore very general. While any explanation must be considered tentative and incomplete, there is indirect evidence that the diminution in Q_{O_2} with time at high temperatures may be attributable to progressive decrease in the intracellular concentration of an oxidative enzyme or enzymes. Thus it is well established that inactivation of many oxidative enzymes by heat becomes appreciable at about 40°C (31). Furthermore, it has been shown that in the bass brain the rate of ammonia production increases with rise in temperature in the range in which oxygen consumption is decreasing. This may imply increased catabolism of some nitrogenous compound important in tissue respiration (19).

It is interesting that oxygen consumption in preparations from rat brain has been observed after more drastic heating than any described above. Thus Cohen and Gerard (9) reported that heating to 60°C did not entirely abolish the oxygen uptake of homogeneous colloidal suspensions of brain in water. We found a small oxygen consumption in suspensions of a dried

data in hand it is convenient to consider the results in the temperature ranges of constant and of decreasing Q_{O_2} under separate headings

1 *Oxygen consumption of cerebral cortex* 0 2° to 37 5°C Mean values of Q_{O_2} of cerebral cortex slices in this temperature range, together with certain derivative statistics, are presented in Table 1

Table 1 *The effect of temperature on the oxygen consumption of brain* 0.2°C to 37 5°C

Temperature (C)	No of animals	Mean Q_{O_2}	Standard Deviation	Standard Error
0 2°	2	0 29		
5 0°	6	0 66	0 134	0 0515
10 0°	20	1 17	0 283	0 0618
15 0°	26	1 76	0 427	0 0824
20 0°	15	2 71	0 546	0 139
25 0°	42	3 78	0 716	0 108
30 0°	30	5 41	0 932	0 170
35 0°	19	7 90	1 528	0 350
37 5°	147	10 21	1 595	0 129

For convenience in comparison with other data (3, 4, 7, 20), the value of mean Q_{O_2} as a function of temperature is presented graphically in Fig 1

It is shown in Fig 1 that the Q_{O_2} is a continuous function of temperature over the range 0 2° to 37 5° Over this range there is progressive increase in the slope of the curve Qualitatively similar curves have been obtained with various organisms and isolated organs on plotting oxygen consumption and many other biological processes as a function of temperature (4, 7, 23, 29) These data, obtained from the largest series of measurements yet made on brain respiration, support the view of Krogh (25) that in general catabolism is a continuous function of temperature up to a maximum and are of course inconsistent with the contrary hypothesis of O'Connor (27)

Except in the earlier reports from this laboratory (19) there are no data on mammalian brain *in vitro* with which to compare these observations The only other recent and relevant findings are those of Himwich and his associates (21) on the oxygen consumption of brain brei over the range 25° to 45°C These data are not directly comparable with ours because of the difference in the method of preparation (12) and the absence of a steady state of oxygen uptake at temperatures as low as 25°C (21) However, when the oxygen uptake reported by Himwich et al for the period 0 to 20 minutes after thermo-equilibration was plotted as a function of temperature over the range 25° to 40°, a curve quite similar to the corresponding segment of Fig 1 was obtained

A curve such as that shown in Fig 1 is probably the resultant of a variety of chemical reactions and physical changes many of which have different temperature coefficients It follows that both metabolic pattern and physico-chemical status are functions of temperature However, for many purposes it is useful to ascertain the over-all temperature coefficient or coefficients

longer exposures at this or higher temperatures resulted in progressively less recovery of the capacity for oxygen consumption when the tissue was returned to a temperature of 37.5°C. The fact that Q_{O_2} leveled off to a steady state at 37.5° after exposure to higher temperatures (below 47.5°) may be explicable on the assumption that a certain fraction of the cell population or of the respiratory mechanism of each cell had been destroyed.

It is evident that when recovery of the initial capacity for oxygen consumption at normal body temperature is used as a criterion, the extent of recovery of excised rat cerebral cortex after exposure to supranormal temperatures depends on the extent and duration of the increase in temperature.

Table 2 Comparison of the oxygen consumption of cerebral cortex slices from a single rat brain suspended in fresh Ringer's and in Ringer's containing heat treated cortical material (See text)

MEDIUM		Q_{O_2}	
Fresh Ringer's		9.74	9.74
Medium A		11.12*	10.65*
Medium B		10.70*	9.29*
Medium C		10.10*	
Medium D		10.35*	
Medium A	2 ml Ringer's containing	50 mgm	incubated brain slices
Medium B	" " "	50 "	" brei
Medium C	" " "	100 "	" slices
Medium D	" " "	100 "	" brei

* Corrected for heat stable respiration of incubated material

It is also clear that by this criterion, this tissue can withstand greater and longer excursions below body temperature than above it. Thus recovery of the initial Q_{O_2} can occur after an hour at 0.2°C. (18) but not after an hour at 40.8°C.

4 *Test for possible production of a toxic factor in brain exposed to heat.* Of the many suggestions offered in explanation of the mechanism of injury of metabolic processes by heat (cf. 4), one is that the metabolic machinery is damaged by an accumulation of metabolites having a "toxic" effect. This conception was tested as follows. In a typical experiment, brain from rat no. 1 was sliced as usual. About half of this sliced material was then ground to a brei in an Hayden grinder. Respirometer vessels were then charged either with slices of brei and shaken for 70 minutes in a water bath at 47.5°C. After this procedure any remaining oxygen consumption would be at the thermostable level.

Cerebral cortex slices from rat no. 2 were then placed in the respirometer vessels containing tissue and medium previously run at 47.5°C. Controls were run by placing cortex slices from rat no. 2 in fresh Ringer's. Measurements of oxygen uptake were then made at 37.5°. Data from a representative experiment are given in Table 2.

of such data, some measurements of Krogh (cited in 23, p 148) on the oxygen consumption of the curarized dog at body temperatures ranging down to 14.1°C were used for comparison. When these data were plotted as log oxygen consumption against Centigrade temperature they could be fitted fairly well by one straight line. The van't Hoff coefficient (Q_{10}) was 2.1. This is quite close to our value of 2.13 for rat cerebral cortex slices over a slightly greater range. Over narrower temperature ranges, values of Q_{10} approximating 2 have been reported for excised mammalian brain by several workers (2, 21, 26).

The significance of the data in the low temperature range with regard to

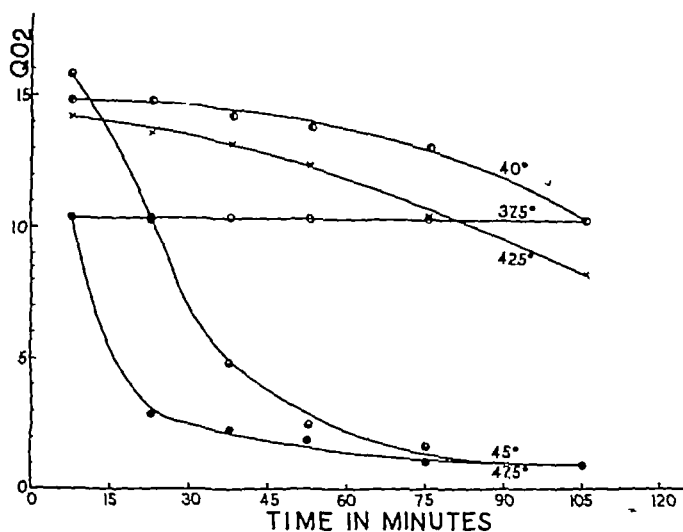


FIG 3 Graph showing diminution of Q_{O_2} with time at high temperature levels

the degree of hypothermia compatible with life in the intact animal and the reversibility of the effects of extreme cold on the oxygen uptake of excised brain have been discussed in an earlier paper (18). The remainder of this paper will be concerned with a consideration of the influence of supranormal temperatures on brain respiration.

2 Oxygen consumption of cerebral cortex 40° to 47.5°C Thirty-five adult albino rats were used in this part of the investigation. The inconstant time course of Q_{O_2} at 40°C has been described. At temperatures above 40° , ranging up to 47.5°C , there was progressive diminution in Q_{O_2} with time, an effect that became more marked the higher the temperature. Thus in this range thermal equilibrium did not involve a steady state with regard to oxygen consumption. This is shown in Fig 3, where values of Q_{O_2} are given as functions of temperature and of time. The data of Fig 3 are taken from the protocol of a representative experiment, all the values of Q_{O_2} presented there were obtained from measurements on cortex slices from a single animal.

per hour (Q_{O_2}) The standard deviation of the distribution and the standard error of the mean were calculated for the values of oxygen consumption at each temperature used in the range 0.2° to 37.5°C

2 It was shown that the graph of log oxygen consumption as a function of temperature in degrees Centigrade was approximately linear over the range 10° to 37.5°C The equation for this curve was

$$\text{Log } Q_{O_2} = -0.2438 + 0.328t$$

where "t" represents the temperature in degrees Centigrade The van't Hoff temperature coefficient, Q_{10} , in this temperature range was 2.13

3 In the range 0.2° to 37.5°C , Q_{O_2} was constant for more than three hours However, at 40° and above, Q_{O_2} diminished with time This effect was the more marked the higher the temperature However, when Q_{O_2} were calculated from the 20-minute period immediately following thermoequilibrium there was no evidence of discontinuity in the Q_{O_2} -temperature curve

4 Decrease in Q_{O_2} with time at 40°C was found to be reversible for periods of exposure to 40° of as long as 3 hours However, at 40.8° an exposure of more than 30 minutes resulted in some loss of capacity for oxygen consumption on restoration to 37.5° This loss became progressively greater with increase in temperature, exposure time or both However, there was a limit to the decline in Q_{O_2} , at least for temperatures up to 47.5° and exposure times up to 2 hours The "steady state" value of Q_{O_2} under these conditions amounted to about 10 per cent of the Q_{O_2} at 37.5° and was termed the "thermostable" Q_{O_2}

5 There was no indication of the accumulation of a substance capable of inhibiting the oxygen uptake of fresh brain tissue when brain slices or brei were incubated for 70 minutes at 47.5°C

6 The possible significance of these data in relation to the effect of hyperthermia on the intact animal was discussed

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powder of brain tissue (heated 24 hours at 105°C) in Ringer's solution This amounted to about one per cent of the respiration of corresponding weights of fresh tissue run at 37.5°C Similar residual oxygen uptake has been observed with suspensions of dried powders of tumor and muscle tissue (13)

3 Reversibility of the inhibition of brain respiration by heat This was tested in the following way In a representative experiment, slices of cerebral cortex from a single brain were placed in a series of respirometer vessels One of these vessels was placed in a water bath at 37.5°C (control), the others in water baths at the higher (experimental) temperatures After the pe-

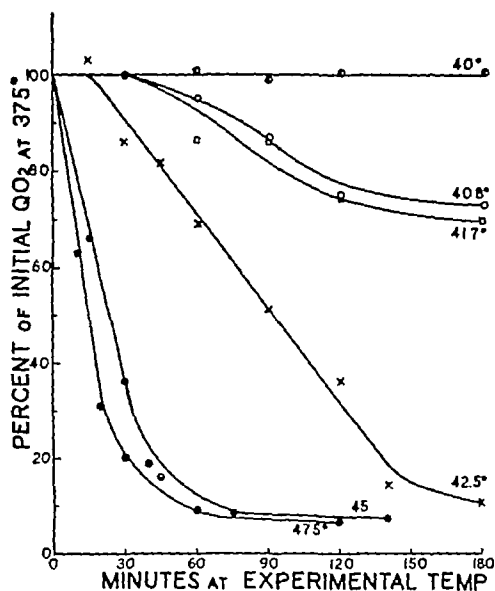


FIG 4 Graph showing reversibility of the decrease in Q_{O_2} at high temperature levels For explanation, see text

riods of supranormal temperature indicated on the X-axis in Fig 4 (minutes from the time the vessels were placed in baths at the experimental temperatures) vessels were removed from the warmer baths and placed in the bath at 37.5°C A value of 100 was assigned to the Q_{O_2} of the control brain slices (maintained at 37.5°C throughout) and the values of Q_{O_2} at other temperatures, given in Fig 4, are in terms of per cent of the control These per cent values were calculated from the measured oxygen uptake during the period 15 to 45 minutes after the experimental vessels were transferred from the higher temperature to 37.5°C For cerebral cortex slices previously exposed to temperatures of 45°C or less, this comparison was satisfactory because after transfer to 37.5°C oxygen consumption remained constant for at least an hour The previous diminution in Q_{O_2} did not continue, and there was no delayed recovery of the type observed by Gray (20) in *Mytilus* gills after heating to 40°C However, for tissues previously exposed to 47.5°C, the relative oxygen consumption values given in Fig 4 were characteristic only of the period 15 to 45 minutes after transfer, because after exposure to this high temperature no steady state of Q_{O_2} was attained on placing the tissue at 37.5°C Evidently processes tending to decrease oxygen consumption which were initiated at 47.5°C continued after transfer to 37.5°C This progressive decrease in Q_{O_2} was limited by the value of the thermostable Q_{O_2}

It is shown in Fig 4 that complete recovery of the initial rate of oxygen occurred after exposure of cerebral cortex slices to 40°C for as long as 3 hours However, after 30 minutes at 40.8°, recovery was incomplete and

EXPERIMENTAL ANALYSIS OF THE FUNCTIONS OF THE BASAL GANGLIA IN MONKEYS* AND CHIMPANZEES

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THE BASAL ganglia are large nuclear masses lying in the forebrain deep to the cerebral cortex, which on account of their anatomical connections and structure have long been regarded as motor in function. Following the disclosures of Fritsch and Hitzig and the later work of Sherrington and many others concerning the functions of the motor cortex, attention was turned to these subcortical structures (6, 7, 24, 25).

The literature dealing with the functions of the basal ganglia (3, 15, 17, 21, 22), however, indicates wide discrepancies between clinical and experimental data, in particular a lack of correlation between recent physiological work on animals and the older clinical concepts of basal ganglial function. Since the reviews just mentioned, particularly that of Bucy (3), are recent and comprehensive, discussion of previous literature will here be omitted.

The basal ganglia dealt with herein are the nuclei caudatus, putamen and globus pallidus. The other motor structures lying deeper,—the substantia nigra, corpus Luysi, reticular substance and nucleus ruber—are usually included in the basal ganglia (8) but in the present study have not been investigated. The data to be reported are based on findings after experimental lesions of caudate, putamen and globus pallidus in 54 monkeys and 6 chimpanzees.

METHOD

This study has been based on observation of pre- and postoperative behavior of primates in which various parts of the basal ganglia had been destroyed via different routes. The observations were largely, although not entirely, on "chronic" preparations. This method was chosen first, because it was one well adapted and long in use in the Laboratory, and second, because it was obvious from the literature that neither by stimulation, by small discrete lesions, such as are made with the Horsley-Clarke instrument nor by clinical means had much positive evidence been uncovered concerning striatal function.

A Surgical procedure. All lesions were made under sterile conditions by a technique previously well established in the Laboratory. An area of the surface of the brain was exposed through skin, bone and dural flap and the lesion made either by blunt dissection from the surface of the brain following hemostasis or by gentle suction within deeper structures. Each of the nuclei of the basal ganglia could thus be removed in whole or in part, singly or together, with one or more of the other nuclei from various approaches as follows:

The caudate was removed by suction most often after excision of area 6, since the head of the caudate lies deep to this cortical area (Fig. 1). It was also sucked out after section of the corpus callosum through the lateral ventricle without injury to cortical tissue. A third method of removal was via the frontal areas after excision of part or all of areas 8–12. In many instances the entire head of the caudate was found at autopsy to have been re-

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It is clear from inspection of Table 2 that there is no indication of the production of a toxic factor when either cerebral cortex slices or brei were incubated for 70 minutes at 47.5°C. Brei was run to test for the possible production of a toxic agent which could not diffuse out of the intact cell (while brei preparation is not standardized, it is probable that few intact cells are present, 28). Double quantities of incubated material (media C and D, Table 2) were used in a second series of tests to decrease the chance that the presence of such a toxic factor is masked by rapid inactivation in fresh tissue. Under such conditions doubling the quantity of toxic agent is more likely to entail reduction of oxygen consumption. This did not occur. In fact, even after allowance for the thermostable Q_{O_2} of the heated material, the Q_{O_2} of the fresh tissue in the presence of the previously heated slices of brei was slightly higher than that of the control in fresh Ringer's (Table 2). If this difference is significant it is probably attributable to the utilization of thermostable nutrients or catalysts contributed by the heated tissue (24).

GENERAL DISCUSSION

Chute and Smyth (8) have shown that possible discrepancy between the rates of brain respiration *in vivo* and *in vitro* is less than formerly assumed, indeed that there may be no discrepancy at all. Hence the data in this paper may furnish a reasonably satisfactory picture of the oxygen uptake of cerebral cortex in the intact rat at corresponding rates of heating, body temperatures and exposure times. However, certain differences are to be expected in view of the difference in milieu, modified by such factors as the presence of the circulation and its progressive failure with rising temperature in the intact hyperthermic animal.

Disappearance of reflex responses and death occur in the intact rat at body temperatures of the order of 42° to 45°C, survival being shorter at the higher temperatures (1, 32). This is the temperature range in which there is progressive decrease in the oxygen uptake of excised rat cerebral cortex. It seems likely that such diminution of brain respiration is an important factor in heat paralysis and death in the intact animal.

Recent work on the frog is in harmony with this view. In this form the outset of heat paralysis, marked by cessation of electrical activity in the brain (14), occurs at about the same temperature as inhibition of brain respiration (6). This would appear to be another instance in which damage to the functional activity of the brain is consequent upon or reflected in depression of oxygen consumption of that organ.

SUMMARY

1. Mean values of the oxygen consumption of cerebral cortex slices from adult albino rats were determined at graded temperatures within the limits 0.2° to 47.5°C. Most observations were made within the range 0.2° to 40°C. Three hundred forty-five animals were used. Oxygen consumption was expressed in cubic millimeters (microliters), N P T, per milligram dry weight

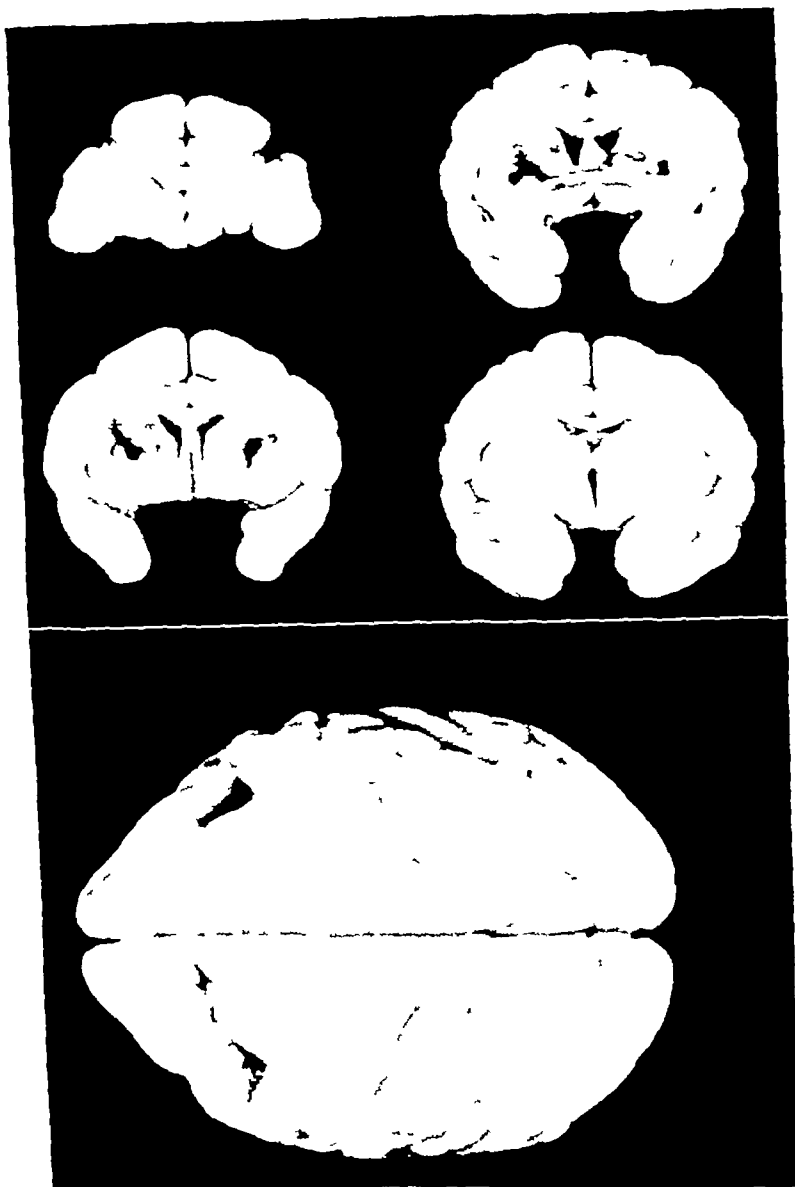


FIG 2 Bilateral ablation of putamen via area 8

it was felt that any additional symptom or symptoms which appeared following combined lesions must be due in part at least to basal ganglia dysfunction. If then, the same symptom could be produced, either by isolated lesion of this basal ganglia nucleus, or by a lesion made via another approach (in which none of the same structures except basal ganglia was touched), the finding could be attributed to destruction of this single area. Since tremor, the most outstanding symptom of lesions of the basal ganglia in this series, was markedly affected by emotion and fatigue the effect of cholinergic and adrenergic drugs was studied

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Tremor was recorded electrically from a number of animals by Dr L F Nims for analysis of its component parts. Electro-encephalograms were made before and after operation in a large series of cases (11, 14)

EXPERIMENTAL DATA

The present investigation has covered a period of nearly six years during which a large number of animals were examined and not until all of this material had been studied was it felt that an anatomical and functional correlation was possible. Two circumstances necessitated this slow progress, first, the relative non-specificity of the striatal nuclei which is such that small lesions anywhere caused either no symptoms at all, or inconspicuous and often transient changes which were shortly compensated for by the remaining tissue. In the second place the basal ganglia lie so near the internal capsule, thalamus and cortex that injury to these structures must always be excluded as a cause of symptoms.

In the following presentation of experimental data the protocols of individual animals have not been given, partly because of the large number examined and partly because this present concept of function is based largely on the entire picture and not on individual instances. In a previous preliminary report (13) abstracts of representative protocols of some of these animals are given. Because of species differences which became apparent during the investigation, the data for the monkeys will be given separately from those for the chimpanzees.

MONKEYS

A Symptoms characteristic of ablation of individual nuclei

1 *Caudate nucleus* In three instances, the head of the caudate nucleus was removed at first unilaterally and then bilaterally through the lateral ventricle after section of the corpus callosum. In no instance was a clear cut postoperative symptom evident. Postoperative activity was normal, there was no paresis, spasticity or tremor. In all three cases area 6 was subsequently removed. Two animals then developed spasticity and marked tremor, the third exhibited the spasticity and reflex grasping which are signs of removal of area 6, but without tremor. At autopsy the caudate lesions in this last case were small. In two of these cases, as in several with lesions of putamen, it was thought that after ablation there was a slight and transient diminution in resistance to passive manipulation of the contralateral extremities. In two other cases the head of the caudate was removed bilaterally through holes made in the cortex of area 8,—the field representing conjugate eye deviation. No symptoms developed here which could be attributed to the caudate lesion. The monkeys showed the fixed gaze, stooped posture, lachrymation and pseudohemianopia characteristic of bilateral area 8 lesions (10), followed later by motor hyperactivity.

Unilateral and bilateral extirpations of area 6 and caudate were made in 18 monkeys. All developed characteristic changes in motor performance. A unilateral ablation of this sort produced contralaterally the transient paresis,

moved without injury to adjacent structures, but the tail and amygdaloid nucleus have never been destroyed

The putamen can be almost completely destroyed through the frontal areas or from area 8 (Fig 2) It can also be reached from the depth of the lateral parts of the crater made by excision of area 6, and it was this approach which was used when combined lesions of caudate and putamen were desired Another approach was from the base of the brain just rostral to optic chiasm and anterior commissure, where it can be reached only about 2 mm deep to the gray matter of the orbital surface by a small hole made lateral to the olfactory bulb From a fourth direction through the depth of Sylvian fissure both putamen and globus pallidus could be destroyed without injury to internal capsule (Fig 3)

The globus pallidus was reached either via the base of the brain or the Sylvian fissure



FIG 1 Bilateral ablation of area 6 and caudate nucleus

as with the putamen In a number of instances either the external segment or both segments of this nucleus were injured without damage to surrounding structures However, we never accomplished an isolated lesion of the internal segment of the globus pallidus

The ansa lenticularis was also sectioned either through the foramen of Munro after the operation devised by Meyers (16), or from the base of the brain through a small hole made just rostral to the chiasm

The Horsley-Clarke apparatus was used for the first ten cases of lesions of putamen and globus pallidus combined with cortical ablations *

B Methods of observation Most of the study of function of the basal ganglia was made entirely through clinical observation Since the effects of lesions of the various parts of cortex through which an approach to basal ganglia was made were already well known,

* The Horsley-Clarke lesions were made by Dr M H Brown and the findings have been reported elsewhere (2)

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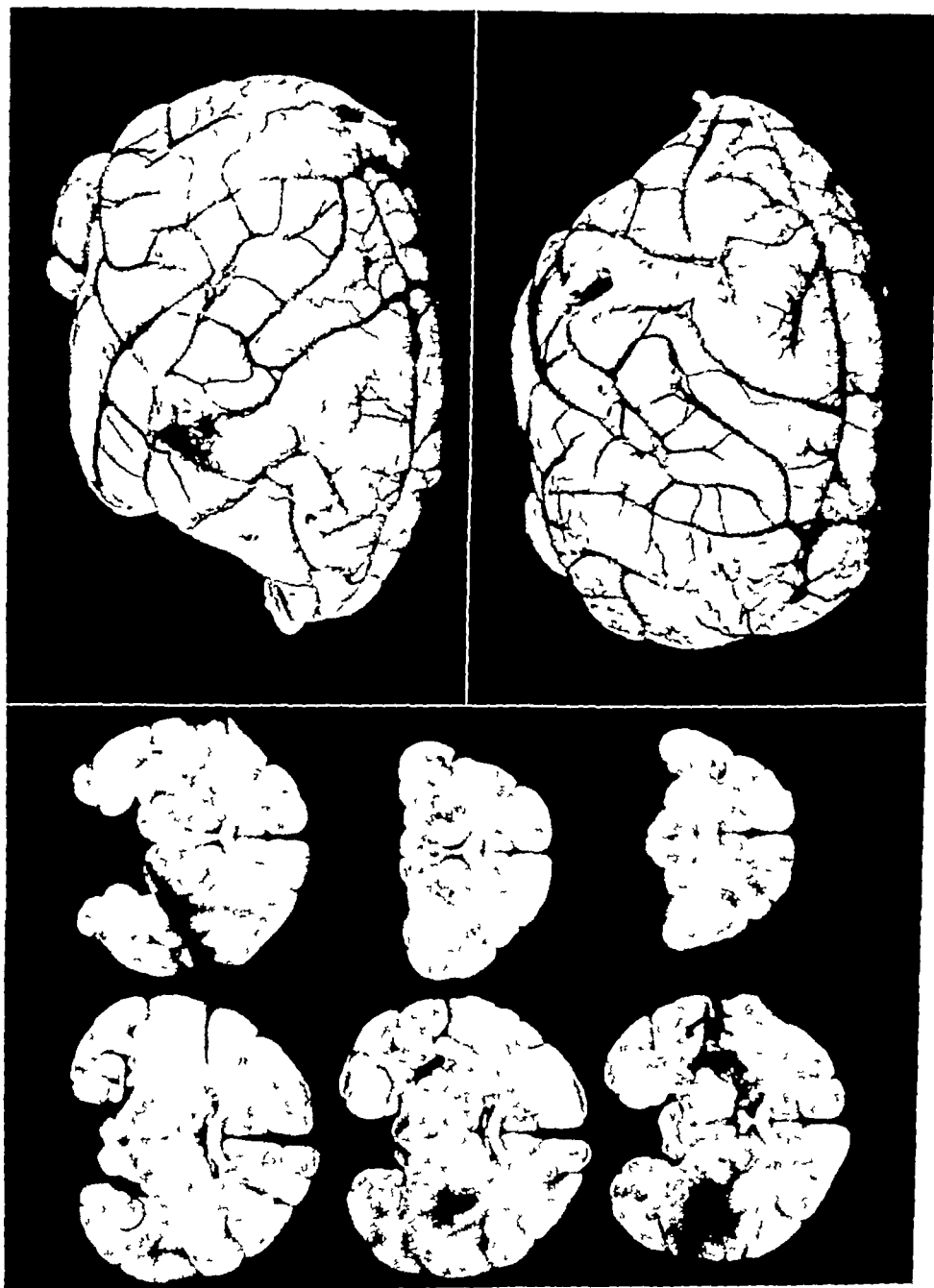


FIG 3 Bilateral ablation of putamen and globus pallidus via Sylvian fissure

spasticity Similar results were obtained from two lesions made via the orbital surface

4 *Section of ansa lenticularis* Since this tract is known to be the chief efferent from all three nuclei of the basal ganglia, its section should cause symptoms similar to those which follow lesions of the basal ganglia This was true of the five animals in which this tract was sectioned Three of these had had previous ablations from area 6 and caudate In all five, a tremor and incoordination of movement which was relatively gross appeared or, in those with previous lesions, became intensified In all, spasticity and paresis were present in moderate degree In all the internal capsule had been damaged Tremor, after a unilateral section of the ansa was always slight and bilateral, spasticity and paresis were contralateral and more severe

In summary, tremor is a constant finding in monkeys when lesions of any one of the three basal ganglia nuclei have been sufficiently large to produce any change This tremor, the characteristics of which will be described later, was present bilaterally as a result of unilateral lesions, it was augmented by bilateral lesions and by ablations of Brodmann's area 6 Spasticity did not appear as a result of isolated lesions of basal ganglia, but only when cortical areas concerned with spasticity were damaged in addition However, the spasticity from the combined cortical and subcortical lesions seemed greater and more enduring than that from cortical lesions alone

B *Inconstant findings*

A number of symptoms appeared in one or more of the monkeys as a result of various lesions and are probably significant, especially in the light of the findings in chimpanzees

1 *Epilepsy*, severe and generalized, lasting several weeks after operation, occurred in two cases after bilateral removal of area 6 and caudate, and in a third after bilateral removal of area 8 and putamen In all three instances it was much more severe and enduring than following cortical lesions in this laboratory The EEG was recorded in two of these monkeys and was characteristic of epilepsy In several other animals typical epileptic attacks appeared on the EEG record although no clinical signs were ever present

2 No true *chorea* was seen in any monkey, but irregular involuntary jerking movements of head and contralateral limbs while at rest were almost always present for a period of hours or days after emergence from the operative anesthetic These choreiform movements appeared in all four of the baboons operated upon and were exceedingly marked in one of them, which may be significant of a species difference as will be shown in the discussion of the chimpanzees The same type of movements were recorded as "marked" in three of the 12 mangabeys and in three of 38 *Macaca mulatta* used Similar movements, although slight and transient, were seen in the majority of the animals with basal ganglia lesions regardless of the site of the lesion within these nuclei

3 *Atrophy* of the contralateral limb musculature, persistent and rather

spasticity and reflex grasp of the area 6 lesion. In addition there developed, frequently but not always, an ipsilateral tremor lasting only a few days and slight at best. Immediately after bilateral lesions these monkeys displayed pronounced bilateral tremor which appeared with the return of voluntary power, was greatest during the first postoperative weeks but persisted in some cases many months after operation. Spasticity following this combined operation was, possibly, slightly greater than when cortical injury to area 6 alone had been sustained.

Examination at autopsy showed that these cases had had symptoms which varied in severity directly with the size of the caudate lesion. Small lesions gave slight symptoms or none at all. There was no indication of focal representation within the nuclei. Arm, head and leg were equally affected and there was bilaterality of function, although the side opposite the most recent lesion was always most severely affected. After the unilateral extirpations it seemed probable that the slight ipsilateral tremor was a manifestation of the bilaterality of this symptom upon which had been superimposed a contralateral paresis.

2 Putamen In seven cases bilateral lesions of putamen or of putamen and globus pallidus were made by the Horsley-Clarke stereotaxic instrument. In none of these did tremor or spasticity appear, although when lesions of area 6 were added (5 cases), spasticity developed in all and tremor in the three suffering the greatest loss of striatal tissue.

Of four bilateral extirpations of putamen made via area 8, three showed marked tremor without spasticity. The fourth case, had relatively small lesions. The putamen was reached and large ablations made from it and from the external segment of the globus pallidus through the depth of the Sylvian fissure of four animals. All developed tremor, and increased resistance to passive manipulation following large bilateral ablations. No symptoms followed any unilateral ablation via this approach.

Excision of large parts of the putamen from the orbital surface of four animals caused similar symptoms except that, the tremor lasted longer since these lesions were deliberately made larger. In all ablations of putamen via any approach, if tremor and spasticity were present, they seemed to be more pronounced in head and arms than elsewhere. This is the only indication of local division within the nuclei so far noted. Bilateral putamen lesions seemed often, but not always to be followed by a *tight closure of the jaw*, and some retraction of the head, together with tremor. Unilateral lesions were followed by deviation of the jaw toward the side of the lesion and by an exaggeration of jaw movements in chewing.

3 Globus pallidus In three animals isolated lesions were made bilaterally by Horsley-Clarke method in the globus pallidus and in three others the lesion was combined with putamen destruction. In none did symptoms appear until subsequent removal of area 8 caused enduring spasticity and tremor. When large lesions were made by suction through the Sylvian fissure, however, there was pronounced tremor always accompanied by

D *Characteristics of tremor*

The involuntary irregular athetoid or choreiform movements present to a slight degree in monkeys were much more pronounced in chimpanzees and analysis of these movements will be added with the chimpanzee data. Analysis of the tremor was attempted in the monkeys by various means

1 *Clinical characteristics* Observation of this tremor in the large series of monkeys proved that it was a tremor of activity. Although totally absent during complete rest, it was often present when there was no active movement, i.e., when the limb musculature was "set" in a state of partial contraction either just prior to movement or as a part of the maintenance of posture. The tremor was always greatest during voluntary movement and especially at its initiation. It was, furthermore, always augmented by tension, excitement, or fatigue. Although it was largely, if not entirely a tremor of action, it differed from the cerebellar "intention tremor" which always became exaggerated toward the end of a movement, this tremor was greatest at the initiation of movement and diminished at the end of the desired act. Often the tremor appeared to precede a voluntary movement.

The "voluntary" or purposeful element of the movement which produced tremor always appeared essential. Partially paretic animals whose limbs moved in association or reflexly, as in reflex grasp had no attendant tremor until, with recovery, a voluntary control of the extremity could be exerted. The bilaterality of function of basal ganglia and the lack of localization of function within the nuclei is almost complete with regard to tremor. Thus, a large unilateral lesion in caudate and putamen (caudate alone is not enough), in putamen, or in putamen and globus pallidus would be followed by bilateral tremor of slight or moderate degree which could be increased at a second operation by removal of either contralateral areas from basal ganglia or of area 6 from cortex.

The close relation of the extrapyramidal motor cortex to basal ganglia has already been discussed (13) but it should be here reemphasized that, tremor does occur in monkeys from which area 6 has been removed simultaneously and bilaterally. Although less in degree it is otherwise closely similar to that which appears following combined cortical and subcortical lesions.

In every instance the most marked tremor occurred from combined lesions. Removal of area 6 and head of caudate, or, more particularly of areas 6, 8 and head of caudate caused maximal symptoms, although the symptoms following large bilateral lesions in basal ganglia alone were almost comparable in severity. By contrast, lesions of area 4, through paresis, always diminished the tremor temporarily, as has been known to be the case in man when Parkinsonian tremor has been abolished by superimposed hemiparesis.

The paroxysms of tremor occurring with movement might appear in any one of several characteristic patterns. For purposes of examination and comparison tremor was clinically classified in four degrees of severity as

marked, was noted in four monkeys having had bilateral (seriatim) removal of area 6 and caudate. Many others with the same lesion showed no such atrophy, and no explanation of the cause can be offered here. However, in all four cases there had been rather marked paresis of some week's duration preceding the appearance of atrophy.

C *Incidental symptoms resulting from damage to adjacent structures*

The following symptoms occurred in a significant number of cases, but were in each instance attributable to injury of structures other than basal ganglia.

1 *Motor paresis* appeared, of course, when lesions had been made in either, areas 4 or 6. It also occurred incidentally if the internal capsule was impinged upon at operation. The placing responses were used as an index of injury to corticospinal tracts during life and verification made at autopsy. If placing responses were present and area 6 undamaged no true paresis was ever seen and hence none can be attributed to basal ganglia deficiency.

2 *Spasticity and postural changes* never occurred when lesions were restricted to the basal ganglia. The jaw-clenching and retraction of the head occurred only when there was known involvement of the putamen and usually of tracts lateral to it leading from face areas 8 and 6. This matter will be discussed later.

3 *Symptoms of lesions in area 8* were those of deviation of the head and eyes toward the side of the lesion, forced circling, in that direction and pseudohemianopia following unilateral lesions. When bilateral, the animal exhibited characteristic fixity of gaze, set face, and stooped posture. Here, as in the relation of area 6 to caudate and putamen, it is the impression that, although no such symptoms appeared after restricted subcortical lesions, addition of these lesions to those of area 8 produced much more intense and persistent postural and behavioral changes.

4 "*Parkinsonian syndrome*" A combined bilateral lesion of area 8 and caudate, of areas 6, 8 and caudate, or of these together with putamen all produced something comparable to Parkinsonism in man. Such animals, sedentary, stooped and with fixed gaze, exhibited tremor and increased salivation. Usually, however, this picture was of only a few weeks' duration, as the symptoms of area 8 ablation characteristically clear rather rapidly.

5 *Hyperactivity* This is also a symptom of bilateral interference with areas rostral to the motor cortical areas,—either are 8 or areas 9–12 (12). It appeared 12 times in these 54 cases. In each case there was evidence of injury to these cortical regions. In six instances there had been deliberate destruction of some or all of the frontal cortex, in the remaining six these areas or their tracts had been encroached upon during operation as indicated by alterations in conjugate deviation of the eyes.

One additional animal may be cited as a control. Operated upon early in the series it developed intense hyperactivity following what was thought to be bilateral destruction of face area 6 and caudate head. At autopsy it was apparent that the sucker had passed in extruding, "lateral to and below" the caudate and that this nucleus was therefore untouched on either side.

6 *Autonomic symptoms* were carefully watched for, because of the early concept that the corpus striatum was concerned with autonomic function. None was found which could be attributed to the basal ganglia alone. There were neither changes in skin temperature nor rectal temperature following operation. Pulse and respiratory rate were likewise unaltered.

While attempting to destroy the globus pallidus from the orbital surface there was inadvertent encroachment upon the hypothalamus in two cases, in both of which pathological sleep developed (18) and was so profound that the animals could be nourished only with difficulty since, when fed, they fell asleep before the food could be swallowed. Pronounced piloerection followed bilateral extirpation of area 6 and lachrymation appeared with lesions of area 8. Multiple gastric ulcers were present at autopsy in two instances, one following bilateral area 6 and caudate extirpation, the other after bilateral removal of area 8 and putamen.

other extremities. The head also, during feeding, could initiate a paroxysm. Once begun, it always increased and then continued sometimes gradually subsiding after 30 seconds or more, but more often lasting as long as the movement. When pronounced, it was sufficiently severe to prevent the accomplishment of the act, or, if the animal was clinging or climbing, to shake it loose from its hold.

As can be seen from Fig. 4 tremor always appeared immediately after operation irrespective of whether paresis was present or not, but during the next few weeks it always increased, to diminish during the third or fourth week to a minimal tremor which was present during the remaining months of life. Three animals with such pronounced tremors were kept for a period of more than two years in order to observe any further changes. None occurred.

2 *Oscillographic records of tremor* The character of the tremor was examined in twelve animals by means of records taken with a Grass ink-writing oscillograph. Electrodes were either inserted into the muscle or fastened to the skin above. The investigation was carried only far enough to determine that when tremor occurred in a limb, it appeared in all groups of muscles of that limb, that its rate for an individual animal was constant at 8–12 per sec and that it was paroxysmal, appearing first in a few muscle bundles, spreading rapidly to involve them all, and then subsiding in reverse order.

3 *Effect of drugs* One most interesting characteristic was the relation of tremor to emotion or fatigue. A monkey showing moderate tremor might be sitting normally in its cage and moving without trace of tremor until shown an attractive bit of food, or threatened with capture. The animal would then immediately begin to shake, and, in reaching for the food, its ability to complete the act without too much interference from the tremor would be inversely proportional to the avidity of its desire. Fear, rage and pleasure all create the same effect. Fatigue in addition greatly accentuates this.

For this reason, the effect of various drugs on tremor was examined in six monkeys. As would be expected, adrenalin, ephedrine, and benzedrine all increase it, but atropine and the cholinergic drug, doryl, definitely diminish it. Both ephedrine and adrenalin, in fact, given in large doses to the unoperated controls, caused a slight but similar tremor to appear temporarily.

4 *Electroencephalographic studies* The effect on EEG of lesions of the basal ganglia have been studied rather extensively and reported elsewhere (11a, 14). Briefly, all evidence pointed to great interdependence between the cortical and subcortical motor areas. Lesions of basal ganglia permanently and markedly altered the electrocorticogram, while complete decortication, in turn, changed the character of the EEG of both caudate or putamen. In a significantly large number of cases, epilepsy, either clinical or subclinical, appeared on the record after combined cortical and subcortical lesions.

5 *Effect of ablations elsewhere in central nervous system* Ablation of area 4 was found to eliminate tremor from a previous injury to basal ganglia for

shown in Fig 4 The most severe type (4+), as indicated in the figure by the height of the symbol, was that which was present at all times except during sleep or complete relaxation, and augmented during movement A less intense degree of tremor (3+) occurred only during voluntary movement, slightly less (2+), was that present only at times and especially during stress and movement The least severe grade (1+) was that which appeared only occasionally, under stress and was confined to the extremity used Paresis was similarly charted in degrees of 1 to 4 plus

The nine cases shown in Fig 4 were selected from groups having different

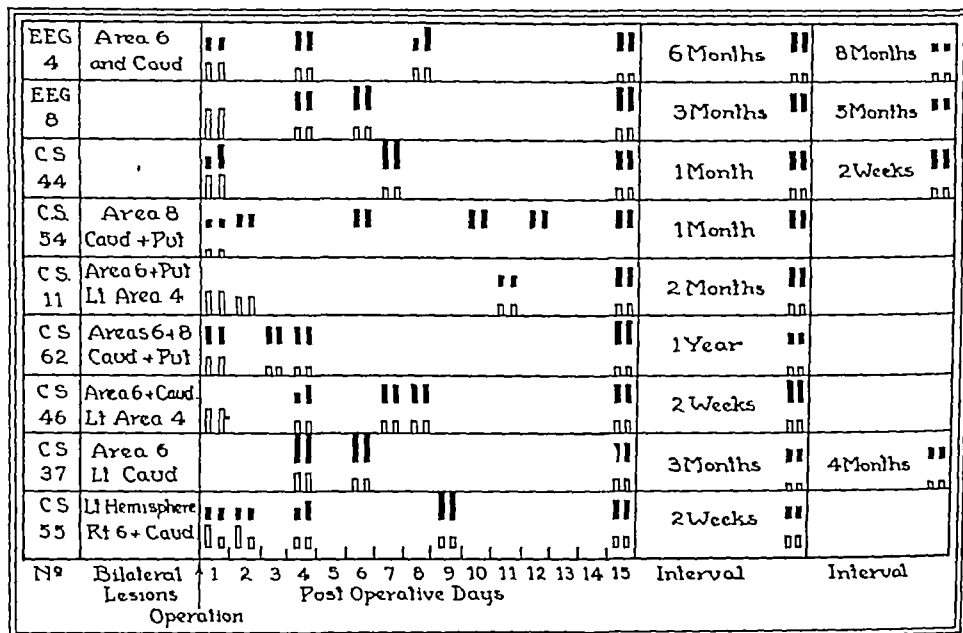


FIG 4 Degree of tremor following various bilateral ablations of basal ganglia nuclei Black indicates tremor and light columns paresis These are recorded in degrees from 1 to 4 plus Note that tremor is at times masked by paresis, but that tremor always reaches maximum at end of second week and then gradually decreases to a permanent minimum

lesions and in these groups, because of the long term during which they lived after operation Amount of tremor and paresis has been indicated only at intervals in order to reduce the chart to comprehensible size From it can be gathered the relation of tremor to paresis and the duration and severity of both symptoms The left and right side have been charted because, when bilateral ablations were made seriatim, the increased paresis of the recently operated side affected the tremors

The tremor had characteristic frequency of about 8-12 per sec which was faster in the smaller animals than in the large It always began in the distal portion of the limb and spread, when at all marked, to the body and

the only true chorea seen Following the second operation and as the paresis disappeared, a pronounced bilateral tremor became obvious, which lasted the remaining months of its life

The fourth and last chimpanzee was also permanently partially incapacitated following removal of the second area 6 and caudate Chorea in this animal lasted only two days but the tremor which appeared after the second operation was undiminished for the remainder of life (3 months) and involved all four extremities as well as head Resistance to passive manipulation was so marked that the animal was almost rigid All movements were slow and accompanied by pronounced tremor, which as, in the monkeys, was exaggerated by emotion or fatigue In this animal, it was constantly present except during sleep No one could have doubted the resemblance of this preparation to that of a Parkinsonian human

DISCUSSION

The signs and symptoms of destruction of the basal ganglia which have appeared in the monkeys and chimpanzees of this series are clearly those of incoordination of the more complex voluntary and involuntary movements This is to be expected from previous knowledge of the tremors, choreas and the like which are seen in man following affections of the same regions The direct functional relationship between the extrapyramidal motor cortical areas and the basal ganglia has also been confirmed in monkeys and chimpanzees as it had been previously in man A third fact shown in earlier clinical material has here been effectively illustrated, namely that there must be at least two mechanisms acting within the basal ganglia complex For, whereas tremor occurred bilaterally and invariably in both monkeys and chimpanzees after disturbances of basal ganglia, choreoathetosis has been only contralateral and has appeared almost exclusively in the chimpanzees

That clear-cut and enduring Parkinsonism or chorea could not be infallibly produced is disappointing It can be explained in two ways First, it is possible that the other basal ganglial structures such as substantia nigra or corpus Luysi must also be involved to produce a permanent effect For, as the most pronounced tremor or chorea appeared only when the cortical level of the extrapyramidal system was involved together with basal ganglia, so the addition of a part of the system at a lower level might cause still further changes

A second and more probable reason, in the light of the present investigation, lies in a species difference Only transient choreiform movements appeared in macaques, they were definitely more pronounced in the mangabeys, which have a larger more highly organized cortex, even more evident in the baboon and so pronounced as to be called true chorea in the chimpanzees, although, even here, the symptoms did not compare in severity to those seen in man

An interesting and significant anatomical correlation of this species difference has been recently discovered by Harman (9) Measuring the cubic

as long as paresis persisted. When, with recovery, the limbs again became used to any degree in voluntary movement the tremor reappeared.

Because of the intimate relation between tremor and emotion the effects of bilateral ablation of the frontal association areas were studied. Tremor was uninfluenced by this procedure. Similarly bilateral removal of temporal lobes had no effect. Two monkeys which had had unilateral ataxia as a result of section of the superior cerebellar peduncle had area 6 and caudate removed bilaterally. Both ataxia and tremor were then present and clearly distinguishable by the individual characteristics.

CHIMPANZEES

A study of the signs exhibited by the six chimpanzees which had lesions of basal ganglia offers more significant data than that from the monkeys, since in every case both choreiform movements and tremor appeared. In each the severity of symptoms and their nature lay between that found in the monkeys and that seen in man. Two experiments were incomplete because of the animals' ill health. One of these had serial ablation of frontal association areas, caudate and putamen. Following the first operation removing left areas 9-12 of cortex together with the head of caudate and much of the putamen there were involuntary irregular twitches of head and right arm for about 48 hours. There were then no signs of motor abnormality whatsoever. The second areas 9-12, caudate and putamen were then removed. Following this there was again a brief period of involuntary twitching, but no paresis, and then a fine tremor which appeared about three days after operation and continued till death two weeks later from diarrhea. The second of these animals, ill before operation and chosen for experimental trial because of this, had area 6 and caudate removed from first one side and then the other. Following the first procedure there were contralateral involuntary twitches of a few days' duration. It died immediately after the second.

Of the four animals with completely successful operations all had brief transient contralateral choreiform movements and more enduring bilateral tremor. The operation in each case was removal of area 6 together with the underlying caudate head and in one instance, a part of the putamen. The variation in degree and duration of symptoms was probably due largely to the variation in the boundaries of the cortical lesion. For, one animal in which area 4 as well as 6 was removed had such severe paresis that both chorea and tremor were masked. Another having minimal symptom had very small lesions both of cortex and caudate.

The third had the largest lesions of caudate and an additional lesion in each putamen together with removal of area 6 with an eight months interval between operations. After each there was true chorea—lasting about a month in each instance—most severe in the arm, but involving head, and to a lesser extent, the leg on the side contralateral to the lesion. The chorea was, in both instances, so severe as to prostrate the animal for some days. It was

caudate, as related to functions associated with increase of manual dexterity, face and eye movement in the higher forms

This functionally and anatomically related pattern can then be used as a background for understanding the mechanism of choreoathetosis. The concept of Bucy (3) of the neural mechanisms underlying choreoathetosis (Fig 5) can then be adopted with one modification. Bucy implicates both area 6

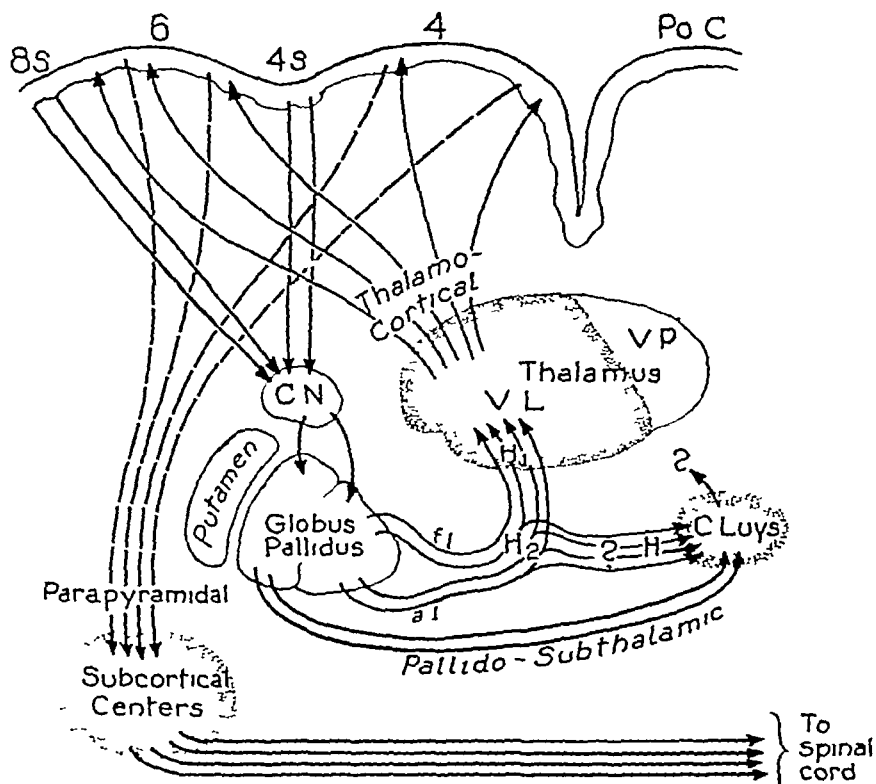


FIG 5 Diagram of pathways for mechanism of choreoathetosis After Bucy, 1942

and the suppressor area of the cortex in the mechanism of choreoathetosis and postulates that interference with suppression (*i e*, caudate) may induce these symptoms. The remainder of the circuit passing from cortex to caudate is via globus pallidus, thalamus and back to cortex. If, however, area 6 is implicated, and if the above indications of localization within the putamen are confirmed, this nucleus also must be included in the circuit, which, when disturbed, produces choreoathetosis, and indeed, it is possible that it is the primary area involved. Since the anatomical interconnections between caudate and putamen are multiple this need not interfere with the concept of Bucy concerning the part played by the suppressor areas in causing choreoathetosis.

volume of the three nuclei of the basal ganglia and comparing them with cortex in a series of primate forms from galago to man he has shown that with an actual increase in volume of the entire complex, there is a relatively great increase in the size of the putamen as compared with either caudate or globus pallidus

Figures for 4 primate forms taken from his table in cubic millimeters are

	<i>Globus pallidus</i>	<i>Caudate</i>	<i>Putamen</i>
Galago	4	21	14
Cercocebus	190	516	567
Chimpanzee	317	949	1628
Man	2353	6192	7472

Dusser de Barenne, Garol and McCulloch (5) by means of strychninization demonstrated that there are functional corticostriatal connections which directly link the cortical suppressor areas 4s and 8s with caudate, whereas areas 6 and 4 are directly connected to putamen * It is therefore not surprising that in the forms in which concomitant with increased complexity of motor function an elaboration of cortical motor areas occurs, there is an enormous relative increase in the nuclei to which these areas send direct connections The greatest change between species occurs between monkeys and chimpanzees, and that between chimpanzees and man, both of which have relatively fine coordination of delicate motor acts such as prehension, there is not so great a change It then becomes entirely possible that the complex involuntary movements of chorea can occur only with disorders of a system normally integrating the complex postural background for such voluntary motor activities

One further impression has been formulated during this investigation which fits well with the concept of the mechanism behind choreoathetosis It will have been gathered from the presentation of experimental data that the relation of the basal ganglia to spasticity is not clear, but that spasticity seemed augmented in combined cortical and subcortical lesions, although absent when ablations were confined to basal ganglia Further more, spasticity, deviation of the jaw and, in bilateral preparations, clenching of the jaw, occurred following injury to cortex and caudate or putamen, but the most pronounced symptoms appeared following large lesions in putamen Choreoathetosis in every instance, affected arm and head, and to much less degree, the leg This is well known to be the case in man In addition it is known both from stimulation and ablation experiments that the functions of area 6 are concerned more with arm and head, than with leg, and that area 8 whose boundaries cannot always be defined lies adjacent to area 6 It is easy then to conceive of a large, extrapyramidal integrative system involving areas 8 and 6 of cortex and the underlying putamen, possibly also

* More
the suppress

(8a) has found a large non-myelinated connection from the
and 8s to the basal ganglia

Even in the absence of direct corticostriatal connections there are enough elements in this pattern of activity to account for the functions of the basal ganglia and even to explain them. The earlier view, based on the connections of the motor nuclei at lower levels (ignoring those with thalamus and hypothalamus), must however be discarded as fitting neither anatomical nor functional data.

This tremor which can be induced whenever a sufficiently large number of cell bodies from the extrapyramidal system have been destroyed is bilateral and enormously affected by emotional stress. There must then be present a motor system which has numerous interconnections and which is directly in contact with both the thalamus, receiving station for incoming sensation, and the hypothalamus, integrator of emotional reaction between somatic and autonomic nervous systems. Since the anterior, ventral thalamic connections to areas 4 and 6 are plentiful, the large pathways from thalamus to striatum and back to thalamus are enough to serve in the integration of normal movement with few or no direct fibres to basal ganglia from cortex.

Interruption of part of the system at any point might then cause symptoms. This could account for the tremor which appears following lesions restricted to thalamus which have been seen clinically. If interference with part of this system yields disorders of movement by disturbing the balance of what remains, it should follow that destruction of the whole system or blockage at some point of the entire circuit should fail to induce such symptoms. This, in fact may be what occurs clinically when tremor or athetosis are diminished or stopped by cortical excisions (3) or by section of efferent from the globus pallidus (16). It may have occurred in the monkeys in two instances. In each, the animal had had extirpations from area 6 and caudate bilaterally and had recovered from the symptoms of spasticity and tremor so that only a minimal degree of the latter remained. In each case subsequent removal of large parts of putamen and globus pallidus from each side resulted in the disappearance of tremor. No such effect appeared however after bilateral section of what was supposedly all of the ansa lenticularis, for this resulted in marked tremor in the three instances in which it was performed.

The tremor of the monkeys and chimpanzees had many of the characteristics of the Parkinsonian tremor of man. Indeed, in the severe stages of postoperative motor disturbance both monkeys and chimpanzees closely resembled these humans. Movements were slow, accompanied by a tremor, augmented by excitement. And, when areas 8 and 6 were also extirpated, there were the fixed face and stooped posture which were undoubtedly added to the picture. All degrees between this and a minimal amount of tremor in action could be seen during the stages of recovery of one monkey.

There is a type of tremor appearing in man which is like that seen in the slight stages of the monkeys and chimpanzees. It is occasionally observed in patients as a tremor unaccompanied with other symptoms, and has been related to disturbances of basal ganglia. It has recently been seen in marked form in a patient who at autopsy showed gross infiltration of caudate and

Figure 6 is a diagram representing the pathways which may underly the mechanism of tremor. Recent anatomical work, notably by Ranson *et al* 19, 20 has emphasized that numerous interconnections exist between striatum and thalamus, discarding the earlier belief that the chief connections of the ansa lenticularis are with the spinal levels via the reticular substance. With this in mind, and because all the tracts to and from the basal ganglia are not as yet established beyond doubt (1, 17) it is possible at this point to

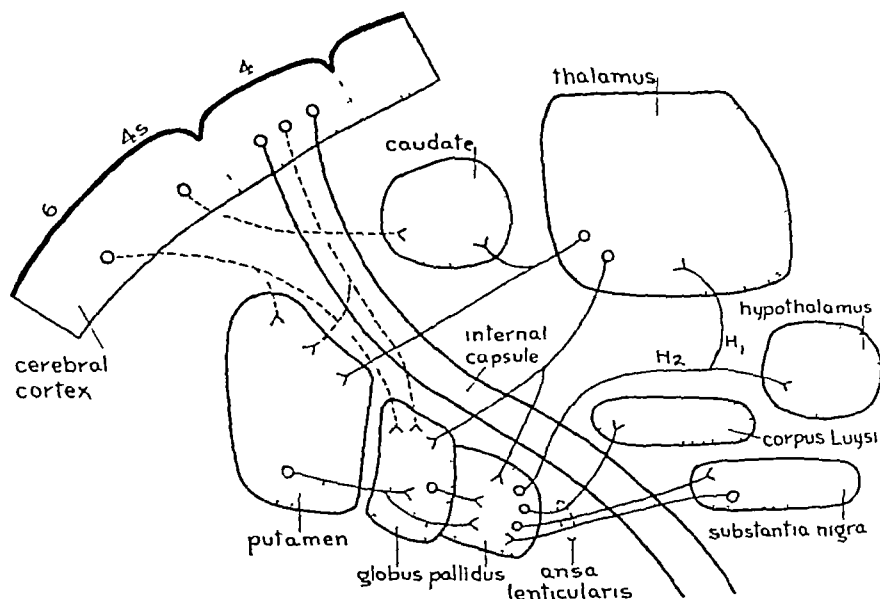


FIG 6 Diagram of mechanism through which tremor, of basal ganglia origin, can be mediated. See text

select those which will fit into the functional picture to add to those of certain existence. Anatomical facts which are certain are these. The interconnections between caudate, putamen and internal and external segments of the globus pallidus are extremely plentiful, there are numerous efferents from thalamus to all these nuclei, the bulk of fibres from the globus pallidus pass via the ansa lenticularis into field H of Forel and thence to the anterior ventral thalamus.

Additional tracts cited by Ranson, but not by all other investigators, are a definite, moderately large tract from globus pallidus to hypothalamus, connections to corpus Luysi and substantia nigra. There is considerable uncertainty about how the red nucleus connects to this system. The cortico-striatal fibres have never been definitely established for although seen by Cajal (4) and others, they have not been found in many instances and must either be few or completely unmyelinated. The evidence from functional anatomy offered by Dusser de Barenne, Garol and McCulloch (5) seems indisputable, as is the more recent anatomical evidences of Gleys (8a).

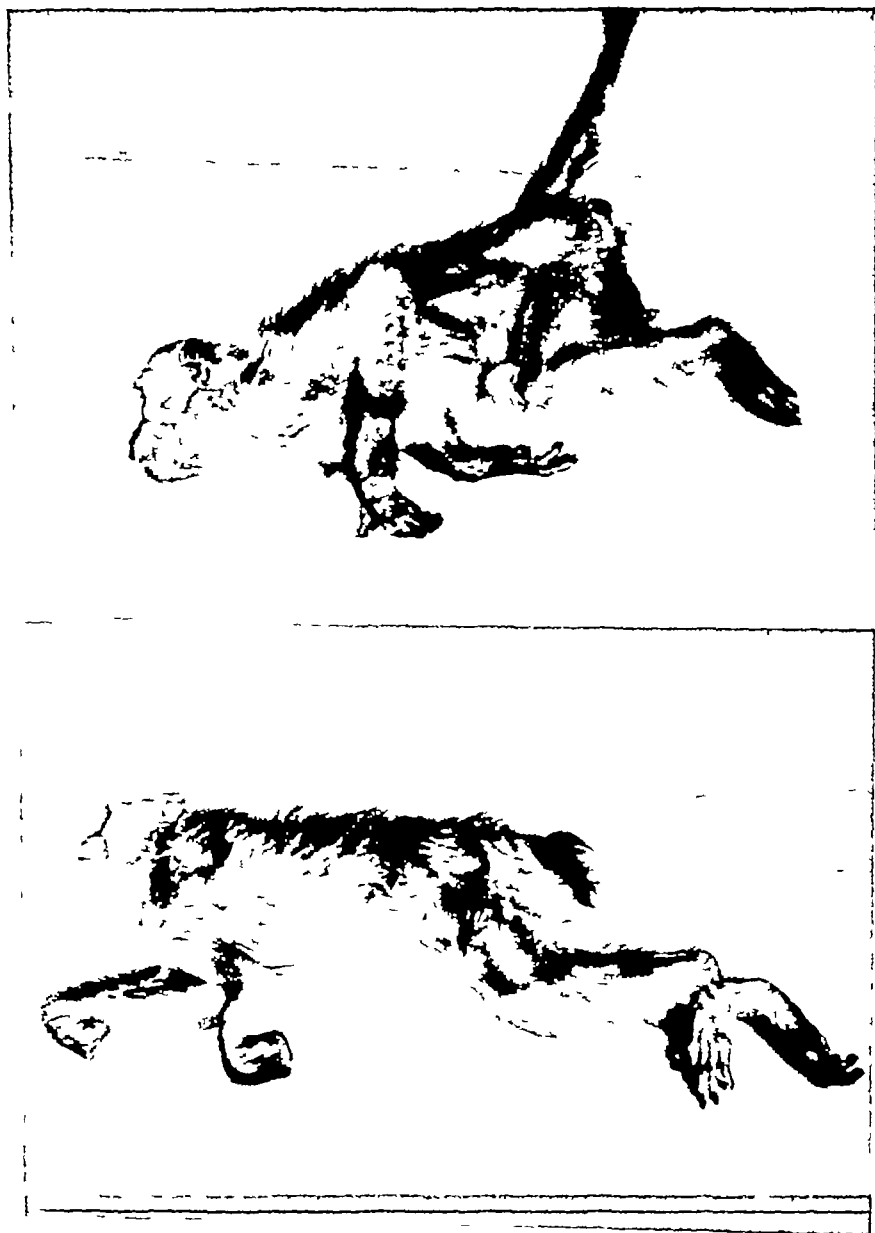


FIG 7 Two monkeys with posture characteristic of bilateral lesions of putamen and caudate in first days after operation. Note retraction of head and extreme flexion of wrist.

putamen of both sides with tumor metastases. It is closely similar to the tremor encountered at some time in all "normal" individuals, a tremor which, absent under ordinary circumstances, appears suddenly during or after strain. The novice surgeon cutting threads for the first time, the "high-strung" individual who, after some crisis is well over, for minutes will shake uncontrollably—these are common examples. This is the type of tremor which appears much more readily in the old even when "normal." Its occurrence to any marked degree in these individuals leads to a diagnosis of arteriosclerotic changes of the basal ganglia. In its extreme degree it becomes arteriosclerotic Parkinsonism.

From the evidence presented here, it is thus possible to postulate an anatomical and functional system integrating the normal background of postural adjustments accompanying purposeful voluntary movements, and that interference with such a mechanism results in choreoathetosis of tremor. The recent analyses of others, especially of Bucy and Ranson, fit closely with this concept.

The question of the relation of posture and of spasticity to the basal ganglia is not as clear and will require further investigation. It has never been known by what anatomical structures the reflexes are integrated which are responsible for the difference in posture between decerebrate and decorticate animals. Some light can be thrown on this matter from present observations. First, as stated above, neither bilateral nor unilateral removal of any parts of the basal ganglia caused any change in posture or motor behavior, but the addition of the basal ganglia to ablations from area 6 frequently caused a resistance to passive manipulation which was beyond that seen from purely cortical lesions. Bilateral extirpation of areas 6 and 8 together with caudate and putamen caused enduring and pronounced spasticity or rigidity, which, in one animal, has been maintained unchanged for more than a year together with tremor. This would not be the case following a purely cortical ablation of area 6.

Second, when all of the frontal cortex together with basal ganglia is removed bilaterally as in two animals, they did not display the same posture or behavior as the controls operated upon at the same time and from which only frontal cortex had been removed. Those animals with destruction of basal ganglia as well as cortex moved less than the controls. The pattern of their movement was less complex. Their posture also differed. Retraction of the head, and extreme flexion of the wrists occurred which are not characteristic of decorticate monkeys. The same picture appeared several other times following second or third operations on monkeys for the purpose of determining the effects of serial removal of basal ganglia nuclei (Fig. 7). These animals had the reflex grasp and postural adjustments to change of position in space of the decorticate animal, although their resting posture was different. They had some of the elements of the extensor posture of a decerebrate monkey, but the same wrist and finger posture. The basal ganglia prepara-

3 Tremor followed the same lesion in chimpanzees and in similar lesions in monkeys. It was an action tremor, absent during complete rest, but present during the maintenance of posture or during movement. It was always marked at the beginning of voluntary movement and was accentuated by emotional stress and fatigue, and was bilateral, appearing whenever a sufficient amount of tissue within the circuit had been damaged.

4 The mechanisms involved in choreoathetosis and tremor predicate two systems not greatly influencing each other, which exist within the basal ganglia. They cannot be geographically distinguished.

5 Anatomical and physiological evidence indicates that the cortical areas 6, 8 and 4s are connected with the basal ganglia and that these nuclei are also directly influenced by thalamus and hypothalamus.

6 The relation of spasticity or rigidity to the basal ganglia has not been made clear by this investigation. But although isolated lesions of basal ganglia do not change resistance to passive manipulation, corticosubcortical lesions seem to increase resistance over that encountered following pure cortical ablations. Some differences in posture also appear when basal ganglia are removed in addition to decortication, as compared to the posture of a pure decorticate preparation.

7 There is a marked difference between the effects of lesions in monkeys and chimpanzees and this is probably true for man also. The difference can be associated with anatomical changes in the putamen and extrapyramidal cerebral cortex. It is concomitant with the development of skilled use of the hands.

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tions exhibit more variety and complexity of spontaneous movement than the decerebrate, although less than the decorticate

The evidence thus points to the basal ganglia as implicated in the maintenance of posture and of resistance to passive manipulation. It is of additional interest that both spasticity and rigidity in man are greatly influenced by emotion and fatigue. The interconnections of thalamus, hypothalamus, basal ganglia and cortex could therefore once again serve as background for a disturbance which is also one of integration of complex postural adjustments and well known to be related to area 6.

The question of differentiation of function of the three basal ganglia nuclei was considered throughout this investigation. No evidence of functional localization has been found. Since the caudate and putamen are structurally one unit and since there are multiple connections between all three nuclei it is not surprising that functional differences have not been disclosed. The dual mechanisms for tremor and for choreoathetosis cannot apparently be geographically separated in monkeys and chimpanzees. In the light of the confusion of data from previous clinical reports and the large amount of effort put into attempted differentiation, it is perhaps not an oversimplification of facts to suggest that in man also the three nuclei act together as a unitary complex.

ADDENDUM

Since this manuscript was completed, the extensive work of Mettler on the effects of unilateral ablations from cortex and basal ganglia has been published (14a). The description of functions found after the various ablations agrees almost completely with that published here and elsewhere (Kennard, 11b). In particular, the increase in resistance to passive manipulation observed when basal ganglia are added to cortical extirpations has been confirmed, as has the transient appearance of jerking, choreiform movements for a few hours or days after operation. The careful analysis of the degeneration following such lesions adds greatly to the value of the physiological observations. Although no such histological investigations have been carried out in the present instance, our cases also, following cortical ablation, showed a decrease in the size of the underlying basal ganglia. Some retrograde degenerative changes were also seen in the cells of both caudate and putamen of such cases.

SUMMARY

1 Lesions of one or more of the nuclear complexes of the basal ganglia of monkeys or chimpanzees produce disorders of movement which are similar to the choreoathetosis and tremor seen in man following lesions of the same regions.

2 Irregular, involuntary, jerking choreiform movements appeared only to any degree in chimpanzees but occurred in slight form in the monkeys also. They were always contralateral to the ablation, affected arm and head more than leg, and were transient.

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THE HIND BRAIN AND THE EARLY DEVELOPMENT OF BEHAVIOR IN FROGS

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(Received for publication April 1, 1943)

THE REFLEX response of a frog tadpole to a tactile stimulus applied to its tail or tail bud changes in nature and duration with development. The response consists of lateral bending of the head and body at the flexure stage, whereas it is a short period of swimming at and after the stage of translatory movements (13). These ontogenetic changes in the nature of the tactile reflex appear to be due to changes in mechanisms within the spinal cord since transection of the cervical cord does not prevent a tadpole from developing to the stage of translatory movements. Transection of the cervical cord does, however, alter the duration of the reflex response. The object of the experiments reported here was to determine the region or regions of the brain responsible for the alteration in the duration of the reflex response and the stage in development at which they first became functional.

MATERIALS AND METHODS

The experiments were carried out on tadpoles of the frog *Rana limnocharis*. The eggs were collected in near-by ponds and hatched in the laboratory. The larvae were fed with algae and cooked liver.

To elicit the tactile reflex a human hair sealed with wax into the tapered end of a glass tube with about 1 cm protruding was used as a stimulator. A light stroke with the hair on the tail bud or tail of the tadpole was the stimulus. The duration of the reflex response, i.e., the interval between the application of the stimulus and the cessation of the reflex response, was measured with a stop watch, which can be read to 0.2 sec.

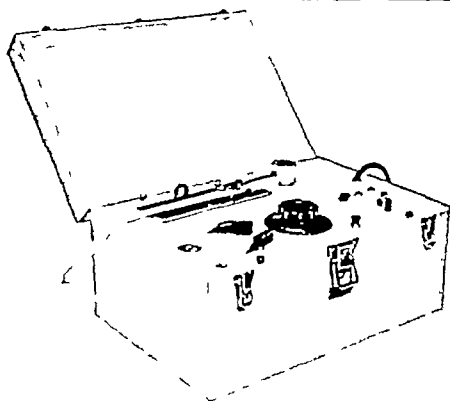
In preparation for operation the embryos were shelled out of the egg into water, which had been previously boiled and cooled to room temperature, to which alcohol sufficient to make it a 2 per cent solution had been added. Larvae were washed for at least 15 minutes in the alcohol-water mixture. The actual operation was carried out on a sterile slide with a sterile iridectomy knife. After the operation, the tadpole was placed in sterile 0.2 per cent saline solution for 1 or 2 hours and then transferred to boiled water cooled to room temperature. The mortality was less than 1 per cent. The operations performed included decapitation, transection of the neural axis at different levels caudal to the hind brain and the removal of one ear vesicle.

In a second series of experiments parabiotic twins were formed after the method of Spemann (11), one of the pair was a decapitate embryo, the other a normal control. Instead of the glass capillary tubes suggested by Spemann we made use of the surface tension of the 0.2 per cent saline solution to hold the embryos together and in place. This change in the method required that the tadpoles be kept in a moist atmosphere to prevent them and the water film from drying.

A third series of tadpoles was treated by immersion in a 1.5×10^{-3} molar solution of KCN. This concentration was demonstrated by preliminary experiments to have no local action on the skin of tadpoles immersed in the solution. Observations were made upon the spontaneous activity of normal, decerebrate, spinal and decapitated tadpoles, before and after immersion in the cyanide solution as well as upon the duration of the reflex response to tactile stimulation. To estimate the amount of spontaneous activity we were obliged to use a very primitive method, i.e., to observe and count the number of times a group of tadpoles moved in a fixed period of time in the absence of external stimuli. No attention was paid to the duration of the movement. The observation time was 60 minutes.

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10 times immediately after it was shelled out and following each 0.5 mm increase (approximately) in length thereafter until it was 6 mm long

The duration of the response is long and variable in the 3 mm embryos (190-250 sec). It decreases in length and range of variation as the body length increases from 3 to 4.5 mm. In embryos between 4.5 and 6.0 mm long it averages 1.5 seconds with little deviation from the mean (Fig. 1). Stating these results in terms of behavioral development instead of body length, the reflex duration to tactile stimulation is short and fairly constant at the stage of transitory movements (at hatching) and remains so up to the commencement of feeding.

2 *On decapitate embryos* In one series the tadpoles were decapitated at the level of the gills—as a means of severing the cervical cord—in the non-motile stage. Thereafter their response to the tactile stimulus was tested at regular intervals until the time at which their controls were capable of maintaining normal spatial orientation while at rest or in motion. This operation—severance of the cord—did not alter the character of the tactile reflex during development.

To determine the effects of cord transection upon the duration of the reflex response it was necessary to limit the observations to acute experiments because decapitate tadpoles usually show signs of deterioration on the second postoperative day. The reflex duration was compared before and after decapitation in each individual. Four series, consisting of 28 embryos each, from the same clutch were studied. They were tested in fours at each 0.5 mm increase in body length between 3.0 and 6.0 mm. Each tadpole was tested ten times before and again ten times 2 hours after decapitation. The

Table 1 The effect of transection of the cervical cord on the duration of the tactile reflex in embryos and larvae of different body-lengths

Series	Spinal tran-section	Body-length						
		2 6-3 0 mm	3 1-3 5 mm	3 6-4 0 mm	4 1-4 5 mm	4 6-5 0 mm	5 1-5 5 mm	5 6-6 0 mm
7/7	Before	3 3"	2 8"	2 7"	2 2"	2 0"	1 6"	1 6"
	After	2 8"	5 6"	46 1"	32 8"	42 5"	21 6"	42 9"
7/9	Before	7 3"	6 6"	2 4"	2 2"	1 8"	1 4"	2 3"
	After	9 8"	40 5"	44 9"	32 4"	72 9"	5 4"	4 5"
7/17	Before	5 6"	9 4"	5 0"	2 0"	1 4"	1 4"	—
	After	11 1"	19 4"	19 4"	11 3"	9 2"	9 5"	—
7/21	Before	8 2"	4 1"	1 8"	1 1"	1 1"	1 2"	1 2"
	After	8 1"	5 2"	9 5"	11 5"	39 7"	6 2"	11 9"
Total averages	Before	6 1"	5 7"	3 0"	1 9"	1 6"	1 4"	1 7"
	After	8 0"	17 7"	30 0"	22 0"	41 1"	10 7"	19 8"
Ratio (after before)		1 3 1	3 1 1	10 0 1	11 6 1	25 7 1	7 6 1	11 6 1

before the cyanide treatment, 15 minutes after it. Beyond this 15-minute period the spinal cord was itself affected. All of the experiments were carried out at room temperature *i.e.*, between 25 and 33°C (mean ca. 30°C).

RESULTS

1 *On normal embryos* In addition to the qualitative changes in the tactile reflex described earlier (13) there is a change in the duration of the

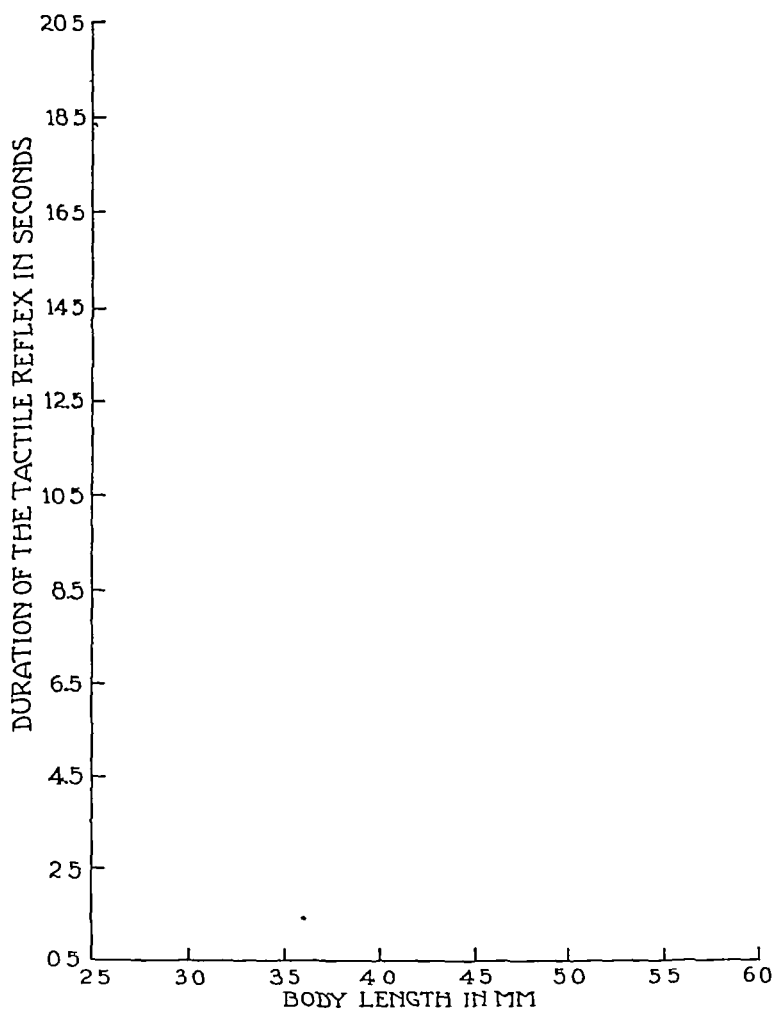


FIG. 1. Showing the change in the duration of the tactile reflex with development in the tadpoles of *Rana limnocharis*.

response. To correlate this change in duration with the development of the tadpoles, two series of experiments were performed. In each series 8 embryos ca. 3 mm long were used from the same clutch. Each embryo was tested

tadpoles with the cervical transection show the marked increase in the duration of the reflex response, but in those with the dorsal cord transection the duration is not lengthened. The spinal mechanism for rhythmic reaction to a single stimulus of short duration appears to be plurisegmental, and the rostral segments seem to play an important rôle in the continuation of the response.

4 *On a decapitate tadpole in parabiosis with a normal* To determine whether or not the release of the spinal centers—as illustrated by their response to a tactile stimulus is permanent—decapitate tadpoles were joined parabiotically to normal controls of the same age and clutch, since they cannot be fed. Of 20 pair of tadpoles so prepared two survived up to metamorphosis. For the first four days after the operation the decapitate tadpole always had a reflex duration 2 to 10 times longer than that of its control partner. Thereafter the duration is about twice that of the normal, which is rather less than that of the response in corresponding acute experiments. The reasons for this difference remain to be discovered.

Near metamorphosis, the fully developed hind limbs of the decapitate twin assume a posture entirely different from that of the normal. The normal twin keeps the hind legs closely flexed to the sides of the body, whereas the legs of the decapitate twin are fully extended. The reflex threshold for the hind legs is much higher in the normal twin than in the decapitated one. The response to tactile stimulation also differs in the twins. A touch on the toe of the decapitate twin elicits simultaneous flexion of the ipsilateral hind leg and extension of the contralateral, this reaction is followed by flexion of the contralateral and simultaneous extension of the ipsilateral hind leg. Alternate responses of this character which correspond to stepping in the adult frog—are repeated 3 or 4 times. In the normal twin, the same type of stimulus evokes but one response: a powerful, rapid, caudal thrust of both hind legs—a fragment of the normal swimming stroke in the adult frog. It is evident that the duration of the induced reflex is longer in the decapitate than in the control twin.

5 *On decerebrate tadpoles and those with the hind brain transected at the level of the ear vesicles* In these experiments the average duration of the response to the tactile stimulus was compared in normal, decerebrate, and spinal tadpoles. In all, four series of experiments were carried out. In each series, 32 embryos from the same clutch were divided into 4 groups. Group 1, served as a control, individuals in groups 2, 3 and 4 were respectively decerebrated, rendered bulbospinal by transection of the hind brain at the level of the ear vesicles and, spinal by section of the cervical cord. All operations were performed at the flexure stage.

About 24 hours after the operation the response to the tactile stimulus of half of each group was tested 10 times, the other half was tested the following day (except in one series, in which all tadpoles were examined on the third day following the operation). The average reflex duration was 1.4 sec in the normal animals (Group 1), 1.8 sec for the decerebrate (Group 2), 7.4

results are summarized in Table 1. In 3 mm embryos the duration is not altered by decapitation, there is some increase in duration at 3.5 mm but an enormous increase follows decapitation in all tadpoles longer than 4 mm. In one extreme case the response lasted 198.7 seconds, whereas the control response continued but 1.8. The results demonstrate quite clearly that the gradual decrease in duration of the response which occurs as development advances in normal tadpoles is due to influences emanating from the higher centers of the brain to the spinal cord.

3. *On larvae with the body transected at the level of the 5th myotome.* The results described above demonstrate that the spinal cord possesses a capacity

Table 2. Showing the difference in the effects of cervical cord transection and of low dorsal cord transection.

Number of experiment	Body-length	Operation	Cervical cord transection	Low dorsal cord transection
1	4 0 mm	Before	2 7"	2 0"
		After	19 9"	2 0"
2	4 2 mm	Before	2 0"	2 3"
		After	26 3"	1 2"
3	4 0 mm	Before	3 2"	1 9"
		After	20 6"	2 0"
4	4 2 mm	Before	1 9"	1 7"
		After	36 9"	2 8"
5	4 0 mm	Before	1 6"	1 8"
		After	17 5"	1 4"
6	4 4 mm	Before	1 5"	1 5"
		After	14 1"	3 1"
Total averages		Before	2 2"	1 9"
		After	22 6"	2 1"
Ratio (after before)			10 3 1	1 1 1

to respond rhythmically to a single stimulus. To determine whether or not this capacity resides in a segmental or plurisegmental mechanism the following experiments were performed. In each of 6 experiments, 8 tadpoles of about the same size and from the same clutch were placed in individual dishes. Each tadpole was tested 10 times with the tactile stimulus. Following the test 4 tadpoles were decapitated at the gills, the bodies of the other four were transected at the level of the 5th myotome. The operations were carried out under a dissecting microscope.

In 2 experiments 4 hours passed between the operation and the time the tadpole was retested 10 times (2 hours were allowed to pass in the remaining 4 experiments before the testing). The data are summarized in Table 2. The

poles described above led us to suspect that normal individuals 4 to 6 mm exhibited less spontaneous activity than spiral ones. To test this suspicion and to obtain corroborating evidence for our conclusions based on the tactile reflex, 4 series of studies on the spontaneous activity of normal, decerebrate, spinal, and decapitate tadpoles, both before and after cyanide poisoning were carried out. The procedures were similar to those outlined above except the

Table 3 Showing the effects of KCN poisoning on embryos and larvae of different body-lengths

Series	KCN poisoning	Body-length					
		3 1-3 5 mm	3 6-4 0 mm	4 1-4 5 mm	4 6-5 0 mm	5 1-5 5 mm	5 6-6 0 mm
6/13	Before	2 9"	—	1 9"	1 2"	1 3"	—
	After	2 6"	—	2 4"	1 7"	3 1"	—
6/16	Before	4 0"	2 1"	1 8"	1 6"	1 0"	0 9"
	After	3 9"	3 6"	2 8"	1 7"	2 2"	1 6"
6/17	Before	3 3"	2 3"	1 9"	0 9"	1 3"	1 1"
	After	4 9"	5 9"	3 0"	2 6"	2 0"	3 0"
6/18	Before	3 4"	1 6"	1 6"	1 4"	1 2"	1 1"
	After	4 4"	3 0"	2 5"	2 6"	1 7"	1 7"
8/1	Before	3 8"	1 8"	1 4"	1 5"	1 3"	1 2"
	After	6 3"	3 9"	2 9"	2 3"	3 0"	2 0"
Total averages	Before	3 5"	2 0"	1 7"	1 3"	1 2"	1 1"
	After	4 4"	4 1"	2 7"	2 2"	2 4"	2 1"
Ratio (after before)		1 3 1	2 0 1	1 6 1	1 7 1	2 0 1	1 9 1

spontaneous activity was tested instead of the duration of the reflex response to tactile stimulation.

In each experiment, the spontaneous activity of all 4 groups (4 tadpoles in each) was observed and recorded for 1 hour. The tadpoles were then placed in a 1:5,000 solution of KCN. The spontaneous activity was then recorded for the 15 minutes immediately following their immersion in the KCN. The total averages of the experiments are presented in Table 5.

Before the immersion in the cyanide, the normal and decerebrate are less active spontaneously than the spinal and decapitate tadpoles. After cyanide poisoning the normal and decerebrate individuals become far more active than the spinal and decapitate ones. The data lead therefore to the following conclusion: the spinal neurons in tadpoles 4 to 6 mm long are constantly inhibited by impulses from the hind brain. When the cord is released from the control of the rhombencephalic centers either by section in the cervical region or immersion in cyanide, an increase in the spontaneous activity re-

results are summarized in Table 1. In 3 mm embryos the duration is not altered by decapitation, there is some increase in duration at 3.5 mm but an enormous increase follows decapitation in all tadpoles longer than 4 mm. In one extreme case the response lasted 198.7 seconds, whereas the control response continued but 1.8. The results demonstrate quite clearly that the gradual decrease in duration of the response which occurs as development advances in normal tadpoles is due to influences emanating from the higher centers of the brain to the spinal cord.

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		After	14 1"	3 1"
Total averages		Before	2 2"	1 9"
		After	22 6"	2 1"
Ratio (after before)			10 3 1	1 1 1

to respond rhythmically to a single stimulus. To determine whether or not this capacity resides in a segmental or plurisegmental mechanism the following experiments were performed. In each of 6 experiments, 8 tadpoles of about the same size and from the same clutch were placed in individual dishes. Each tadpole was tested 10 times with the tactile stimulus. Following the test 4 tadpoles were decapitated at the gills, the bodies of the other four were transected at the level of the 5th myotome. The operations were carried out under a dissecting microscope.

In 2 experiments 4 hours passed between the operation and the time the tadpole was retested 10 times (2 hours were allowed to pass in the remaining 4 experiments before the testing). The data are summarized in Table 2. The

However, we must not overlook the fact that the larval or fetal spinal cord has a simple structure at the early stages. In the spinal cord of the adult frog, especially in that of the adult mammal, there are intra-spinal inhibitory mechanisms. The work of Fulton, Liddell and Rioch (5), and the recent experiments of van Harreveld (12) all tend to show that the phenomenon of "spinal shock" is due to such intra-spinal inhibitory mechanisms.

The inhibitory influence of the hind brain over the spinal neurons matures long before its excitatory action on the same neurons, and even before its segmental motor functions. As demonstrated by our experiments, in the inhibitory control by the hind brain over the spinal cord is exercised in the embryo of *Rana limnocharis* at a body-length of about 4 mm. The most important excitatory control of the hind brain as a suprasegmental apparatus over the spinal cord is the action of the vestibular nuclei on posture and movement. In 3 experiments each with 10 embryos, we extirpated one ear vesicle at approximately the non-motile stage, and observed at which body-length they began to show symptoms of unilateral labyrinthectomy. Such symptoms always appear at 5.0 to 5.5 mm. Breathing through the internal gills and feeding—2 motor functions involving the participation of the segmental apparatus of the hind brain—occur in the tadpoles of *Rana limnocharis* respectively at 5.5 to 6.0 mm and 6.0 to 6.5 mm.

According to Barron's (2) summary of his conjoint work with Barcroft on the behavioral development in the sheep, the fetal respiratory movements are inhibited by the mid-brain from the 40th day after insemination, and general body movements by the basal ganglia from the 55th day onward. Around the 40th day, the righting reflexes which are controlled by the mid-brain, certainly have not reached full development in the sheep fetus. The basal ganglia also are not sufficiently mature to assume excitatory action on the spinal neurons in the fetal life. Even the new-born sheep cannot regulate its body temperature—a function under the control of the basal ganglia.

Since the frog and the sheep are widely separated in the phylogenetic scale, it is remarkable that, in both the frog tadpole and the sheep fetus, the inhibitory action of a higher motor center on the spinal cord precedes in maturation its excitatory action. It may be well asked: Is this a general rule in the development of the central nervous system of all vertebrates? It is an important question worth further inquiry.

As to the exact location of the origin of the inhibitory impulses from the hindbrain, we are inclined to the view that it lies in the vestibular nuclei. Fulton, Liddell and Rioch (5) have demonstrated that the knee jerks of the cat become prolonged in duration after section of the ventrolateral column and also after destruction of the vestibular nuclei. However, Herrick (7) has not found it possible to define in the larval hind brain of *Amblystoma* any nuclei exclusively related to the afferent fibers from the labyrinth. In his classic investigations on the correlation of the development of the central neural axis with that of behavior in *Amblystoma*, Coghill (4) does not explicitly describe the growth of the nuclei of the eighth cranial nerve. Our

sec for the bulbospinal (Group 3) and 9.3 sec for the spinal tadpoles (Group 4). Clearly, groups 1 and 2 are characterized by a short response, groups 3 and 4 by a much longer one. In other words the duration of the tactile reflex is not affected by decerebration whereas it is lengthened markedly by transection of the neuraxis at the level of the ear vesicles and by section of the cervical cord. The centers exercising the inhibitory effect upon the spinal neurons appear, therefore, to be in the rostral half of the hind brain.

6 *After cyanide treatment in normal tadpoles* Cyanide poisoning produces (see appendix) the same effect on the developing as on the adult nervous system. It suppresses the function of nerve cells and fibers, and in the developing nervous system the last elements to mature are the first to succumb. For example, in a 6 mm larva, the functions of the midbrain, the hind brain, and the spinal cord are abolished in that order, the opposite to that in which their functions are established in development.

In 5 series of experiments, we attempted to poison with KCN tadpoles of different body lengths and to observe the effect on the duration of the tactile reflex. In each series 24 embryos of approximately the same length from the same clutch, were equally divided into 6 groups. In one series only 16 were used. They were divided into 4 groups. The six groups were tested respectively at the following body lengths: 3.5, 4.0, 4.5, and 5.0, 5.5 and 6.0 mm. The response of each subject was tested 10 times before it was transferred to an individual dish containing a 3×10^{-3} molar solution of KCN and again 5 times immediately after the transfer. There was no significant change in the reflex duration of the embryos shorter than 4 mm, but a definite, though slight, lengthening in all tadpoles longer than 4 mm.

7 *After cyanide treatment in normal, decerebrate, decapitate and spinal tadpoles* The effects of cyanide poisoning on the duration of the tactile reflex was studied in 4 series of experiments, in normal, decerebrate, decapitate and spinal tadpoles. In each series 64 embryos of approximately the same size and from the same clutch were divided into 4 equal groups. The individuals in group 1 were normal controls, group 2, decerebrate, in group 3 the spinal cord was transected in the cervical region, and those in group 4 were decapitated. The 16 tadpoles of each group were tested, four at one time, at 4 different body lengths (of the normal control) i.e., 4.1-4.5 mm, 4.6-5.0 mm, 5.1-5.5 mm, 5.6 to 6.0 mm, 6.1-6.5 mm. The tests were made and the KCN was administered as described above. The individual results of the series are collected in Table 3.

The animals used in the experiments described in section 5 were also treated with a 1:5,000 aqueous solution of KCN after 10 observations had been made on their reflex duration. Immediately after immersion in the KCN solution they were again tested 5 times. There was a slight but definite prolongation of the reflex response in normal and decerebrate animals after immersion in the KCN but no such lengthening in the low decerebrate, spinal and decapitate ones.

8 *Observations on spontaneous activity* Casual observations on the tad-

The symptoms of paralysis appear a little later. These symptoms vary with the age of the poisoned tadpole. A 6 mm larva first becomes unable to right itself, following this, it loses the control over the direction of swimming, soon it is unable to swim, reacting to tactile stimulation by repeated lateral flexions of the tail, and the last reaction lost is lateral flexion of its tail in response to gentle pressure applied on its head. The only symptom of cyanide poisoning in an embryo in the late flexure stage is the gradual decrease in the number of times lateral bending of the head is repeated in response to tactile stimulation, then it reacts only to gentle pressure applied on its head, and finally it ceases to respond to any stimulation.

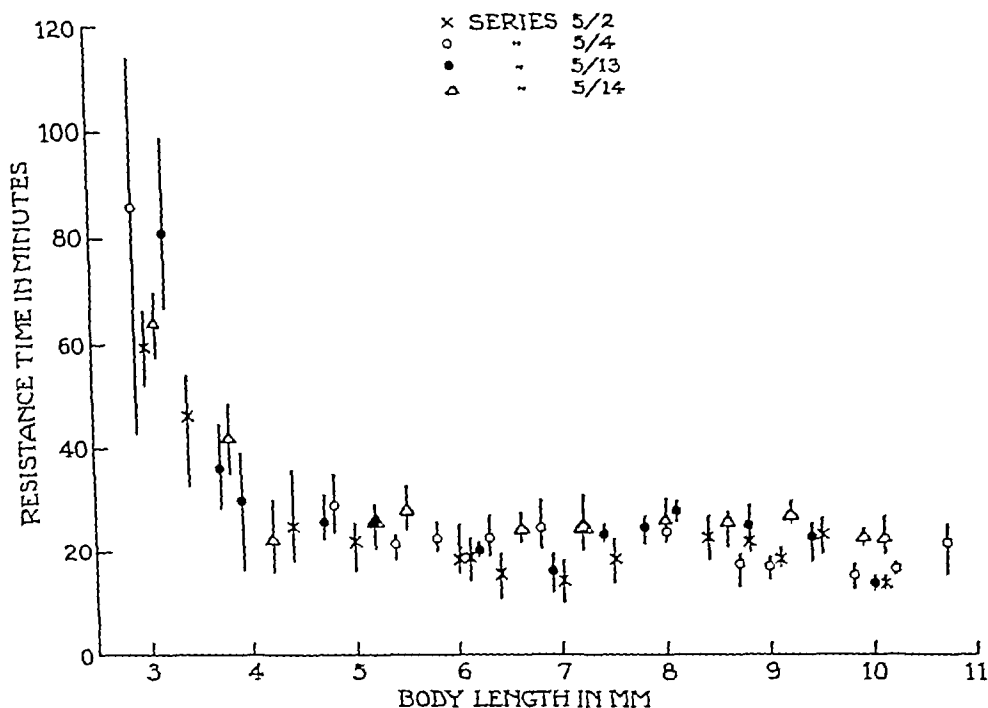


FIG. 2 Showing the variation in the resistance to treatment with cyanide with growth in the tadpoles of *Rana limnocharis*

To summarize, cyanide suppresses the functional activity of the developing as well as of the adult nervous system in the frog. With the developing nervous system, the last structure to mature is the first to succumb to cyanide.

2. *The variation in susceptibility to cyanide with growth in tadpoles.* The younger an embryo, the less susceptible it is to poisons and drugs (9). However, the actual course of variation in susceptibility to cyanide with age in frog tadpoles is not known.

In order to trace the changes in susceptibility with growth, it is necessary to have a standard of measurement. For simplicity, we measured, instead of susceptibility, its reciprocal, the resistance of tadpoles to cyanide poisoning. As a standard of measurement, we adopted the time interval from the moment of immersion of the tadpole in the KCN solution to the moment when it fails to respond to repeated tactile stimulation but still reacts with a lateral flexion of the body to gentle pressure applied on the head. We shall call this time interval the resistance time.

In 4 series of experiments, we measured the variation in the resistance time from the body-length of 3 mm to that of 10 mm. Each series of experiments was carried out with tadpoles from the same batch of eggs. At each test, 8 tadpoles of the same size were used, and they were given the tactile reflex test once per minute. The duration of reflex was not

sults The increase in activity is less after spinal transection than after cyanide poisoning The reasons for this difference are not known

DISCUSSION

Between hatching and the commencement of feeding, frog tadpoles remain relatively inactive and react to external stimulation with but a brief response Either hanging from the surface of water or lying on the bottom of a pond, they appear as if they were inert materials This inertness lessens to a great extent their chance of being detected and preyed upon by their enemies, and has, therefore, a great survival value for such utterly defenceless, small creatures as tadpoles The experiments reported in preceding pages shed some light upon the physiological mechanisms responsible for this inactivity

A spinal mechanism for repeated reactions to a single brief stimulation is the first to mature in the frog embryo This accounts for the long duration of the tactile reflex observed in embryos about 3 mm long A little later, there develops in the rostral half of the hind brain a center which inhibits the spinal mechanism This rhombencephalic center appears to send inhibitory impulses to the spinal neurons, thereby reducing the spontaneous activity and limiting the reaction to external stimulation to a short response from the time of hatching to the commencement of feeding

A spinal mechanism for repeated reaction to external stimuli of short duration appears to be present in mammalian fetuses, and it may persist in their adult life Barcroft and Barron (1) noticed that sheep fetuses with the cervical cord transected are spontaneously more active than normal fetuses, of the same age They state the reflexes of such fetuses "In contrast to the inertness of a normal fetus of 70 days, flexion reflexes *with afterdischarges* were present in both fore and hind legs" (*italics being ours*) The fetus of the sheep with a cervical cord transection, like the beheaded frog tadpole, exhibits considerable spontaneous activity, and reacts to a brief external stimulus with a long train of repeated responses It is a well established fact that all adult mammals exhibit flexor spasms of the hind legs to any stimulation, when they completely recover from the total division of the dorsal cord The flexor spasms, of course, considerably outlast the stimulus Spontaneous flexor spasms also are frequently observed

As to the details of the spinal mechanism for repeated reactions to a brief stimulation, we must confess that little is definitely known The mechanism may be partly extra-spinal each repeated response is evoked by the proprioceptive impulses set up in the muscles by its preceding response The mechanism may be wholly intraspinal One may assume, as Kubie (8) has in the brain, the existence in the spinal cord of a closed circuit in which nerve impulses can travel round and round The collaterals from the spinal motoneurons to the floor plate, as described by Coghill (4) in the brain of the larval *Amblystoma*, may be the possible branches which form the assumed closed circuit But there are at present no experimental facts supporting either of the conjectures expressed above

REFLEX INHIBITION OF INTESTINAL MOTILITY MEDIATED THROUGH DECENTRALIZED PREVERTEBRAL GANGLIA

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THE CELIAC and other prevertebral ganglia (2, 3) differ structurally from the sympathetic trunk ganglia at least to the extent that not all the axons whose terminal branches arborize among the ganglion cells undergo degeneration following section of the preganglionic fibers of spinal origin. Some of the data also support the assumption that section of mesenteric nerves does not result in degeneration of all their constituent fibers distal to the section. The fibers which remain viable in the distal segments of these nerves, following section, have been interpreted as fibers of enteric origin which extend into the prevertebral ganglia. The results of experimental studies (3, 4) also support the assumption that enteric stimulation or direct stimulation of mesenteric nerves may elicit reflex responses through the decentralized inferior mesenteric and celiac ganglia.

Certain investigators have failed to obtain reflex responses through prevertebral ganglia following section of their preganglionic fibers of spinal origin and have questioned the validity of our findings. On the basis of experimental findings in unanesthetized dogs, Youmans, Karstens and Aumann (6) concluded that intestino-intestinal inhibitory reflexes are not mediated through the decentralized celiac or other prevertebral ganglia even though the mesenteric nerves distal to these ganglia remain intact. On the basis of experiments carried out on cats, Freund and Sheehan (1) concluded that the intestino-intestinal inhibitory reflex is abolished by removal of both sympathetic trunks from above the stellate ganglion to the brim of the pelvis. Contrary to these findings, Warkentin, Huston, Preston and Ivy (5), in an experimental study of reflex inhibition of hepatic bile flow in response to distention of the proximal colon in dogs, concluded that the celiac ganglion is either a true or a pseudo-reflex center for this reaction.

In view of the lack of agreement regarding the occurrence of reflex connections in the prevertebral ganglia, it has seemed desirable to carry out further studies bearing on this problem, including both anatomical and physiological experimentation.

MATERIAL AND METHODS

Cats were used as the experimental animals and the operative procedures were carried out with the animals under nembutal anesthesia. In one series the connections of the inferior mesenteric ganglia with the central nervous system were interrupted by bilateral extirpation of the lumbar segments of the sympathetic trunk, section of the celiac roots and section of the hypogastric nerves. These animals were allowed to live 21 days following operation. The inferior mesenteric ganglia were then prepared for study by a modification of Cajal's silver technic. In another series a colonic branch of the inferior mesenteric

experimental technique is not sufficiently refined for an attack on the problem from the physiological standpoint

SUMMARY

1 The reflex response of embryos of *Rana limnocharis* to tactile stimulation varies in nature and duration as development advances. For embryos 2.0 mm body-length the duration of the response averages more than 4 sec. In embryos 3.0–4.5 mm the duration decreases gradually and in larger individuals remains ca. 1.5 sec on the average.

2 Transection of the cervical cord does not alter the nature of the tactile reflex with development, nor its duration in embryos 3 mm long. In larvae longer than 4 mm the duration of the reflex is lengthened by transection of the cord. The increase in duration persists up until metamorphosis.

3 Transection of the dorsal cord does not increase the duration of the tactile reflex in 5 mm tadpoles.

4 Decerebration does not alter either the nature or duration of the tactile reflex in tadpoles at any stage. Destruction of the rostral half of the hind brain has the same effect as transection of the cervical cord.

5 In the early stage of its action cyanide poisoning lengthens slightly the duration of the tactile reflex in normal and decerebrate larvae but does not alter it in spinal and bulbospinal preparations.

6 Spinal tadpoles exhibit more spontaneous activity than normal and decerebrate ones. Cyanide poisoning in the early stage of its action increases the spontaneous activity of normal and decerebrate tadpoles but does not alter it significantly in spinal larvae.

7 These data indicate that a mechanism is laid down in the spinal cord of *Rana limnocharis* in its development, from the non motile stage to one of transitory movements, for repeated reactions to a brief tactile stimulus and that this mechanism is inhibited by impulses from the rostral half of the hind brain at and after hatching.

APPENDIX

ACTION OF CYANIDE ON THE DEVELOPING NERVOUS SYSTEM OF THE FROG

In the course of the cyanide poisoning experiments described in the main paper, we had to inquire into the following two questions. Firstly, is the action of cyanide on the developing nervous system the same as that on the adult nervous system? Secondly, does the susceptibility of the tadpoles vary with growth? The results of our inquiry are presented in the following paragraphs.

1 *The action of cyanide on tadpoles.* The first symptom appearing within 1 or 2 minutes after a tadpole has been immersed in a 1:5,000 aqueous solution of KCN is a marked increase in spontaneous activity. This may be due to either irritation of the skin or abolition of the inhibition of the spinal cord by higher motor centers. The following facts indicate the second alternative to be the true explanation. In the first place, the concentration adopted produces no eruptions on the skin, visible either to the naked eye or under the dissecting microscope. Second, embryos at the flexure stage (about 3 mm long), whose hind brains have not matured, show no increase in spontaneous motility after the administration of cyanide. Finally, spinal tadpoles in contrast with normal and decerebrate ones do not increase their spontaneous activity after cyanide, as reported in the main paper.

teric arteries, these fibers must be regarded as the axons of enteric ganglion cells. They also resemble fibers of enteric origin within the enteric plexuses both in caliber and in the absence of myelin. In view of the persistence of terminal branches of axons in the prevertebral ganglia following interruption of the preganglionic fibers of spinal origin, it may be assumed that some axons of enteric origin reach the prevertebral ganglia via the mesenteric nerves.

Physiological In preparing the animals for acute experimentation on the large intestine, the colon was transected approximately at the level of the inferior mesenteric artery and the recording balloon placed in the proximal

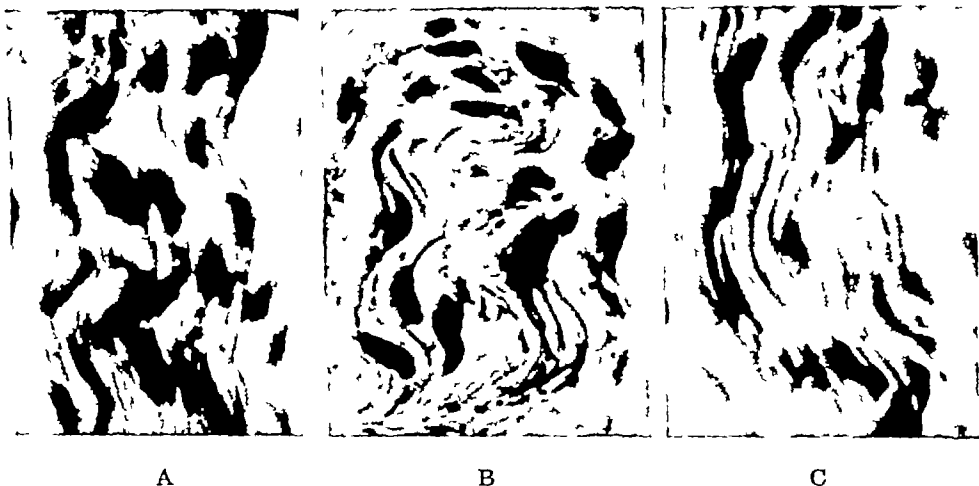


FIG 1 Photomicrographs of sections of proximal (A, C) and distal (B) segments of colonic nerves in a cat taken 40 days after transection of these nerves. The nerve fibers appearing in B are those which have remained viable following degeneration of those which grow distalward.

segment immediately after removal of the spinal cord from the lower cervical region caudalward. In most of the animals used distention of the distal segment of the colon with air or water under pressure equal to 200 cm of water or over or direct faradic stimulation of nerves to this segment, while the proximal segment was undergoing spontaneous contractions, resulted in inhibition of this motility and usually in lowering of the tonic level in some degree (Fig 2A). In most of the trials motility was resumed promptly, in others inhibition continued for variable intervals following cessation of the stimulation.

In the chronic experiments the stimulation trials were carried out in the same manner, but 7 days or longer after decentralization of the inferior mesenteric ganglia by bilateral extirpation of the lumbar segments of the sympathetic trunk and section of the celiac roots and the hypogastric nerves. In these experiments it proved advantageous to sever the pelvic

recorded. The test was repeated until the resistance time of each tadpole had been determined. The results of the 4 series are presented in Fig. 2. In this figure, the average resistance time obtained at each test with the range of deviation as indicated by a vertical line is plotted against the body-length. At 3 mm, the resistance time averages 80.6 minutes, from 3 to 5 mm, it gradually decreases, and from 5 to 11 mm, it remains stationary at an average of 20 minutes. The fall in resistance time from 3 to 5 mm is exponential, as verified by plotting on semilogarithmic paper.

The high resistance of young embryos to poisons and drugs is a fact well known but little understood. The exponential fall in resistance from the flexure stage to approximately the stage of transitory movements is probably correlated with the development of the spinal cord. This conjecture is supported by our experiments (13) on the effects of transection of the cervical cord on the development of behavior in frogs, and also by the classic study of Coghill (4) on the growth of the larval spinal cord in *Amblystoma*. Unfortunately no similar investigation on the development of the spinal cord of frog tadpoles has been carried out, and consequently no direct comparison can be made between the curve of resistance time and that of the growth in the spinal cord. The constant resistance time from 5 to 11 mm is probably due to the fact that the larval spinal cord has attained its first phase of growth after the stage of transitory movements.

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the inhibitory response in the proximal segment of the intestine to direct faradic stimulation of more distal mesenteric nerves occurred repeatedly, but the inhibitory response to distention of an extensive segment of the intestine usually could not be elicited more than a few times in succession, particularly when pressures greater than 200 cm of water were used. In some animals this method of stimulation failed after the first trial. In a few

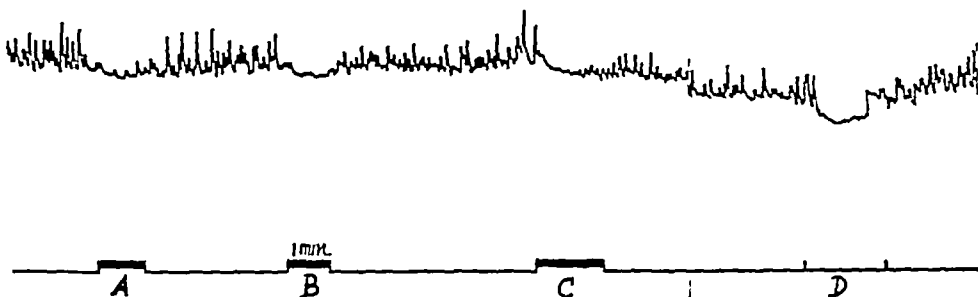


FIG 3 Kymographic records of motility in the proximal segment of the transected jejunum of a cat with the spinal cord removed from the lower cervical region caudalward. A, B, and C show inhibition elicited by faradic stimulation of mesenteric nerves to the ileum. D shows inhibition elicited by distention of the ileum.

instances distention of a segment of the intestine with air resulted in air embolism and immediate death of the animal.

COMMENT

The anatomical data in the present investigation in general corroborate the earlier findings of Kuntz (2, 3) which indicate the persistence of axon terminations in the prevertebral ganglia following interruption of the preganglionic fibers of spinal origin, and the persistence of intact fibers in the distal segments of mesenteric nerves following transection of these nerves and the arteries with which they are associated. The 21-day interval allowed for the degeneration of the terminal branches of interrupted axons in the inferior mesenteric ganglia probably is sufficient to insure complete degeneration of all the interrupted fibers. This study of intraganglionic axon terminations has been limited to the inferior mesenteric ganglia because of the relative certainty that all the preganglionic fibers of spinal origin which reach these ganglia are interrupted by the operative technic employed. Complete decentralization of the celiac ganglia by splanchnic nerve section or extirpation of the sympathetic trunks, without injury to the celiac plexus, is more difficult. The 40-day interval allowed for the degeneration of the fibers growing distalward in the distal segments of the mesenteric nerves undoubtedly is sufficient to insure complete degeneration of all nerve fibers separated from their cells of origin. The persistence of unmyelinated nerve fibers in the distal segments of transected mesenteric nerves indicates the occurrence of fibers, in these nerves, other than those which grow distalward.

artery, the nerves associated with it and an intestinal branch of the celiac artery with its associated nerves were ligated at two points close together and transected between the ligatures. These animals were allowed to live 40 days following operation, after which the distal and proximal segments of the arteries and nerves in question were prepared for study by the protargol technic.

In the series of cats used in acute physiological experiments, decentralization of the prevertebral ganglia was accomplished by removal of the spinal cord from the lower cervical region caudalward. This method was adopted because of the certainty with which it interrupts all preganglionic fibers of spinal origin. In some of these animals the vagus nerves also were interrupted just below the diaphragm by transection of the esophagus and adjacent structures. In a series prepared to determine whether the intestino-intestinal inhibitory reflex can be elicited through the inferior mesenteric ganglia following degeneration of the preganglionic fibers of spinal origin these ganglia were decentralized by bilateral extirpation of the lumbar segments of the sympathetic trunk and section of the celiac roots and the hypogastric nerves. These animals were permitted to live one week or longer before the physiological experiments were carried out.

In the experiments involving the response of the proximal portion of the colon to stimulation of the distal portion, a rubber balloon was placed in the proximal portion and connected with a tambour for kymographic recording. In those involving the response of the proximal portion of the small intestine to stimulation of more distal mesenteric nerves the balloon was placed in the jejunum. In all experiments the intestine was transected distal to the recording balloon in order to interrupt the enteric plexuses. In most of the experiments the mesenteric nerves were stimulated by distention of a segment of the intestine distal to the transection with air or water under moderate pressure. In others faradic stimulation was applied directly to the corresponding mesenteric nerves.

EXPERIMENTAL DATA

Anatomical The results obtained in the preparations of inferior mesenteric ganglia in which the preganglionic axons of spinal origin had undergone degeneration corroborate in general the earlier findings of Kuntz (3) in similar material. Most of the axons whose terminal branches arborize among the ganglion cells and ramify among the dendrites in dendritic tracts and glomeruli disappeared, but some remained intact. Terminal boutons in contact with ganglion cells were observed only rarely, but terminal branches of axons in intimate relationships with ganglion cells and in dendritic tracts and glomeruli were seen in all of the sections.

Preparations of the distal segments of mesenteric nerves both to the colon and the small intestine taken after degeneration of the nerve fibers growing toward the intestine, following section of a branch of the inferior mesenteric or of the celiac artery with the nerves associated with it, exhibited numerous intact fibers (Fig. 1B). These fibers are not equally abundant in all the nerve fiber bundles associated with the artery. Some bundles appear to be entirely devoid of intact fibers. Sections of the scar tissue in which the distal ends of the proximal segments and the proximal ends of the distal segments of the interrupted artery and nerves are imbedded afford no conclusive evidence of regeneration of the interrupted nerve fibers. Regeneration of these fibers probably was prevented by the ligature proximal to the level of the transection. No regenerating fibers could be traced through the scar tissue. The viable fibers in the distal segments of the interrupted nerves, consequently, do not represent regenerating axons. In the absence of ganglion cells in the mesentery associated with the distal branches of the mesen-

siderably greater pressure. The exact threshold of stimulation has not been determined, but the pressures used in these experiments (200 cm. of water or over) were well above the threshold intensity in most instances. In some cases distention with pressures equal to 200 cm. of water failed to elicit a reflex response.

The results of the acute experiments in the present series afford conclusive proof that the intestino-intestinal inhibitory reflex, following decentralization of the prevertebral ganglia, is mediated through these ganglia, but they do not indicate whether the reaction is a true reflex or a pseudo-reflex. The results of the chronic experiments in which distention of the distal segment of the colon caused inhibition of motility in the proximal segment in animals in which the inferior mesenteric ganglia had been decentralized 7 days or longer previously afford conclusive evidence on this point. Pseudo-reflex activity carried out through branching visceral afferent fibers of spinal ganglion origin is precluded in these experiments since the visceral afferent fibers which reach the colon via the inferior mesenteric plexus, like the pre-ganglionic fibers of spinal origin to the inferior mesenteric ganglia, had undergone degeneration. Synaptic contacts in the inferior mesenteric ganglia effected by collateral branches of local ganglion cells are not precluded, but there is no reason to assume that stimulation of efferent terminal structures in the colon by distention of this viscus results in the conduction of impulses centralward in efferent axons. In view of the anatomical demonstration of intact fibers in the distal segments of colonic and other intestinal nerves following degeneration of the interrupted fibers growing distalward in them, it appears more probable that impulses rising in the colon, under the conditions of these experiments, are conducted into the inferior mesenteric ganglia through axons of enteric ganglion cells which effect synaptic connections in these ganglia and thus constitute components of true reflex arcs.

Enteric ganglion cells whose axons extend into prevertebral ganglia do not conform to the accepted definition of visceral afferent neurons and should not be classified in this category. They are essentially similar to the receptive components in the reflex mechanisms within the wall of the enteric canal. The latter effect synaptic contacts in the ganglia in which they are located or in adjacent enteric ganglia, the former effect synaptic contacts in prevertebral ganglia.

SUMMARY

In the cat the terminal branches of some axons remain intact in the inferior mesenteric ganglia following degeneration of all preganglionic fibers of spinal origin which reach these ganglia. Some nerve fibers also remain intact in the distal segments of mesenteric nerves to the colon and the small intestine, following their interruption, after degeneration of all the fibers which grow distalward. These are regarded as the axons of ganglion cells located in the wall of the enteric canal which extend into the prevertebral ganglia.

nerves before stimulating the distal colon in order to avoid reflex parasympathetic stimulation of the proximal colon which, in some instances could not be overcome by the reflex stimulation elicited through the inferior mesenteric ganglia. In the absence of intact pelvic nerves, the inhibitory responses in the proximal segment of the colon elicited by distention of the distal segment, following degeneration of the preganglionic fibers to the inferior mesenteric ganglia (Fig 2B), were entirely comparable to those elicited by the same stimulation in the acute experiments in animals in

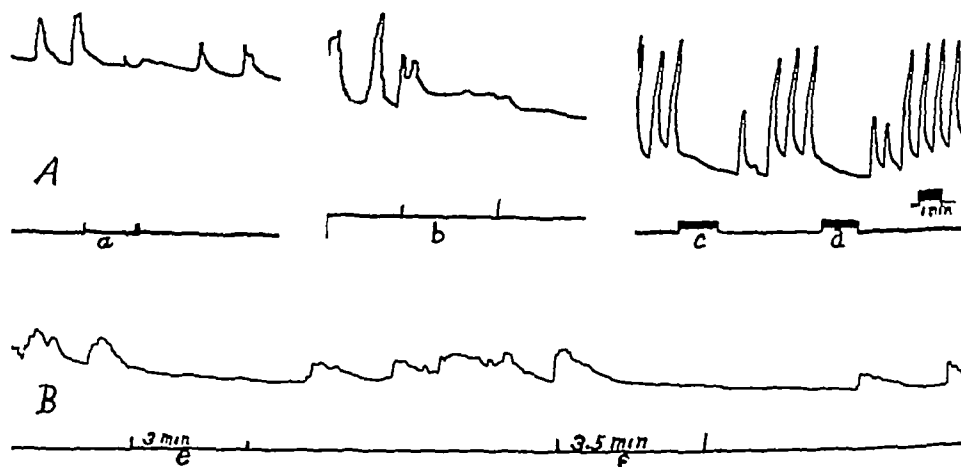


FIG 2 Kymographic records of motility in the proximal segment of the transected colon of the cat. a and b show inhibition of motility elicited by distention of the distal segment of the colon in an animal with the spinal cord removed from the lower cervical region caudalward. c and d show inhibition elicited by faradic stimulation of nerves to the distal segment of the colon in an animal prepared in the same manner. e and f show inhibition elicited by distention of the distal segment of the colon 9 days after decentralization of the inferior mesenteric ganglia by extirpation of the lumbar segments of both sympathetic trunks and section of the celiac roots and the hypogastric nerves.

which decentralization of the inferior cervical ganglia had been accomplished by removal of the spinal cord.

In preparing the animals for the experiments involving the intestino-intestinal inhibitory reflex mediated through the celiac ganglia, the small intestine was transected near the middle of the jejunum and the recording balloon placed in the proximal segment immediately after removal of the spinal cord from the lower cervical region caudalward. Distention of the ileum with air or water under pressure equal to 200 cm. of water or over or direct faradic stimulation of mesenteric nerves distal to the transection, while the proximal segment of the intestine was undergoing spontaneous contractions, usually resulted in partial or complete inhibition of the motility in the proximal segment and lowering of the tonic level in some degree (Fig 3). Section of the vagus nerves was without apparent effect on the inhibitory responses obtained in these experiments. In most of the animals

siderably greater pressure. The exact threshold of stimulation has not been determined, but the pressures used in these experiments (200 cm. of water or over) were well above the threshold intensity in most instances. In some cases distention with pressures equal to 200 cm. of water failed to elicit a reflex response.

The results of the acute experiments in the present series afford conclusive proof that the intestino-intestinal inhibitory reflex, following decentralization of the prevertebral ganglia, is mediated through these ganglia, but they do not indicate whether the reaction is a true reflex or a pseudo-reflex. The results of the chronic experiments in which distention of the distal segment of the colon caused inhibition of motility in the proximal segment in animals in which the inferior mesenteric ganglia had been decentralized 7 days or longer previously afford conclusive evidence on this point. Pseudo-reflex activity carried out through branching visceral afferent fibers of spinal ganglion origin is precluded in these experiments since the visceral afferent fibers which reach the colon via the inferior mesenteric plexus, like the pre-ganglionic fibers of spinal origin to the inferior mesenteric ganglia, had undergone degeneration. Synaptic contacts in the inferior mesenteric ganglia effected by collateral branches of local ganglion cells are not precluded, but there is no reason to assume that stimulation of efferent terminal structures in the colon by distention of this viscus results in the conduction of impulses centralward in efferent axons. In view of the anatomical demonstration of intact fibers in the distal segments of colonic and other intestinal nerves following degeneration of the interrupted fibers growing distalward in them, it appears more probable that impulses rising in the colon, under the conditions of these experiments, are conducted into the inferior mesenteric ganglia through axons of enteric ganglion cells which effect synaptic connections in these ganglia and thus constitute components of true reflex arcs.

Enteric ganglion cells whose axons extend into prevertebral ganglia do not conform to the accepted definition of visceral afferent neurons and should not be classified in this category. They are essentially similar to the receptive components in the reflex mechanisms within the wall of the enteric canal. The latter effect synaptic contacts in the ganglia in which they are located or in adjacent enteric ganglia, the former effect synaptic contacts in prevertebral ganglia.

SUMMARY

In the cat the terminal branches of some axons remain intact in the inferior mesenteric ganglia following degeneration of all preganglionic fibers of spinal origin which reach these ganglia. Some nerve fibers also remain intact in the distal segments of mesenteric nerves to the colon and the small intestine, following their interruption, after degeneration of all the fibers which grow distalward. These are regarded as the axons of ganglion cells located in the wall of the enteric canal which extend into the prevertebral ganglia.

The time interval following section of these nerves may have been long enough for the regeneration of the interrupted fibers, but this apparently was prevented by the ligature of the proximal segment. Careful examination of sections of the scar tissue formed at the site of the transection of the arteries and nerves, furthermore, afforded no evidence of the growth of nerve fibers from the proximal segments of the interrupted nerves into the distal segments. The fibers which have persisted in the distal segments of these nerves undoubtedly represent mainly axons of ganglion cells located in the intestinal wall. On the assumption that such fibers effect synaptic contacts in the prevertebral ganglia, they may be regarded as constituents of mechanisms comparable to the local reflex arcs within the enteric plexuses, the reflex activity of which has been amply demonstrated.

The physiological data in the present investigation are in full agreement with the anatomical findings. They also corroborate the earlier experiments (3, 4) and are in agreement with the finding of Warkentin, Huston, Preston and Ivy (5) that the hepatic bile flow may be inhibited by distention of the proximal colon or direct stimulation of its nerve supply, following decentralization of the celiac ganglia by bilateral section of the vagus and splanchnic nerves and extirpation of the lumbar segments of the sympathetic trunks.

The vagus nerves play no part in the intestino-intestinal inhibitory reflex either before or after interruption of the nerves connecting the celiac ganglia with the spinal cord, consequently, these nerves were not interrupted in all the animals used in this series of experiments. In those in which the vagi were severed below the diaphragm, the experimental results were not modified by this procedure.

Decentralization of the prevertebral ganglia by removal of the spinal cord from above the first thoracic segment caudalward must be regarded as complete, since no sympathetic preganglionic fibers emerge from the spinal cord at higher levels. The inhibitory responses in a proximal segment either of the small intestine or the colon to distention of a more distal segment or direct stimulation of the corresponding mesenteric nerves, therefore, must be mediated through the prevertebral ganglia. This also is indicated by the observation of Kuntz and Van Buskirk (4) that the inhibitory response in the proximal portion of the small intestine is abolished by application of a nicotine solution to the celiac ganglia. Warkentin *et al.* (5) also reported that the inhibition of the hepatic bile flow in response to distention of the proximal colon or direct stimulation of its nerve supply, following bilateral section of the vagus and splanchnic nerves and extirpation of the lumbar segments of the sympathetic trunks, is abolished by application of a nicotine solution to the celiac ganglia.

Under the conditions of our experiments, the threshold of stimulation of the intestino-intestinal reflex mechanisms which persist following decentralization of the prevertebral ganglia was relatively high. They were not activated by distention of a segment of the intestine only sufficient to activate the spinal reflex arcs. Their activation required distention by con-

EXPERIMENTAL HYPOGEUSIA FROM HORSLEY-CLARKE LESIONS OF THE THALAMUS IN *MACACA MULATTA**

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THE THALAMIC termination of visual, auditory and somatosensory fibers is well known and detailed point-to-point projections of these fibers onto the cerebral cortex have been worked out. For three categories of sensation the fact, to say nothing of the locus, of a thalamic relay is largely a matter of inference. These are visceral sensation, labyrinthine sensation and taste.

There are definite indications with respect to the locus of the thalamic nucleus for taste. Bornstein (3, 4) has recently presented evidence that taste is localized in the postcentral operculum rather than in the hippocampal region as is usually stated. Such a localization suggests a different thalamic relay than would be likely if the locus were rhinencephalic. The projection to the inferior end of the cortical somatosensory area arises, according to Walker (15) and Le Gros Clarke (6), from the nucleus ventralis posteromedialis (arcuate nucleus). The secondary trigeminal pathways terminate in this nucleus (16). Therefore, a reasonable hypothesis is that taste impulses also terminate in or near the arcuate nucleus.

Actually what little is known of the central taste pathways favors localization in this region of the thalamus. For example, Adler (1) describes an instance of a glioblastoma of the third ventricle accompanied by heterolateral reduction of gustatory sensibility and cutaneous sensibility of the face. Adler concluded from her histological study of the case that taste is represented in the medial part of the arcuate nucleus. Walker (15), from a consideration of pertinent clinical and anatomical studies, also suggested that the arcuate nucleus is the thalamic relay for taste.

In the first of a series of experiments designed to test this hypothesis clear-cut, positive evidence was obtained in one of three animals (2). In this study further evidence of the same purport is described.

METHODS

The gustatory discriminatory capacity for quinine hydrochloride was determined in 10 immature *Macaca mulatta* monkeys before and after bilateral electrolytic lesions of the posteroventral thalamic nuclei. Testing of taste was accomplished by a preference technique similar in principle to that employed by Richter for rats (12) and Blum, Walker, and Ruch for monkeys (2). The apparatus and method have been discussed in detail elsewhere (11).

* Aided by a grant from the Fluid Research Funds, Yale University School of Medicine. These experiments are part of a thesis presented (H.D.P.) in candidacy for the degree of Doctor of Philosophy, Department of Physiology, Yale University 1943.

A taste deficit is manifested by a postoperative shift of the curve to the right. To state such changes succinctly it is convenient to choose one or more points (thresholds) on the curves for comparison. Two such points have been chosen. The rejection threshold is the lowest concentration at which no more

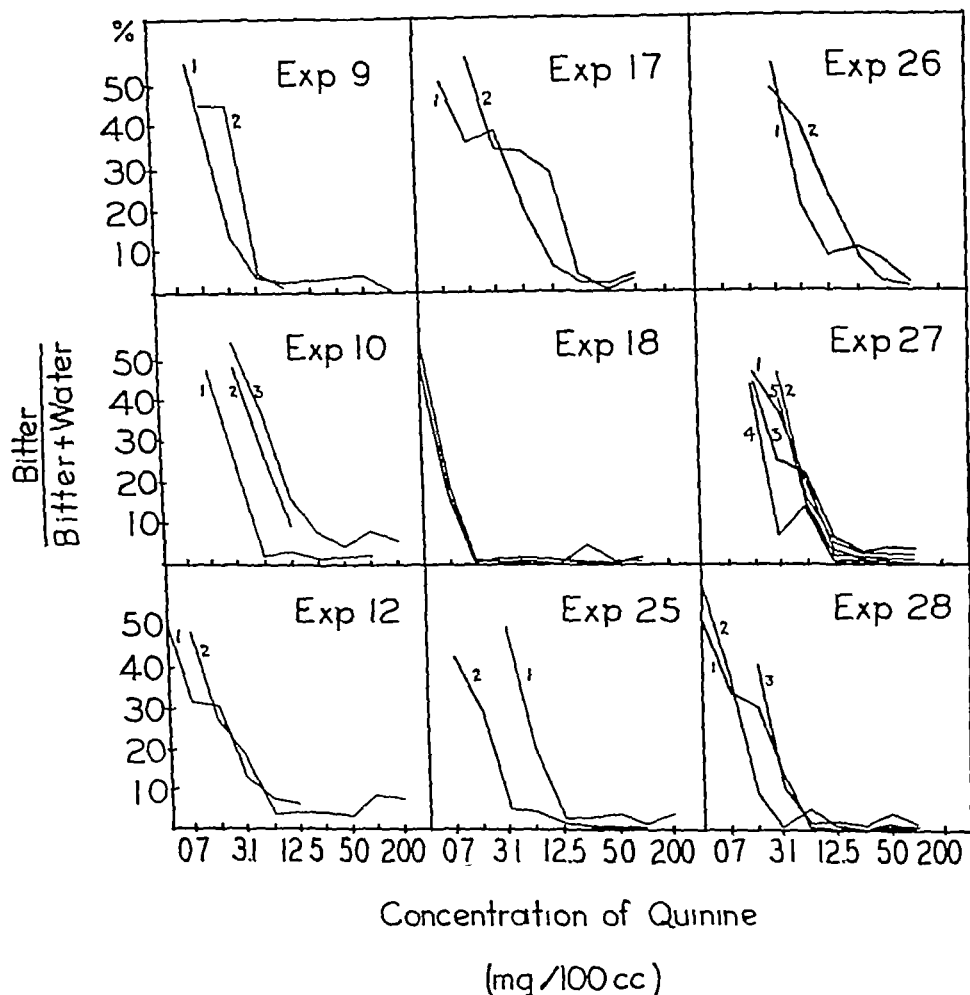


FIG 1 Repeated independent determinations of the threshold curves to test the reliability of the method

quinine is drunk than is allowable for purposes of sampling which we have taken to be 10 per cent. The *acceptance threshold* is the concentration at which the curve approaches the 50 per cent line where chance rather than bitterness determines how much of each solution is drunk. Because of chance fluctuation a zone of 10 per cent on either side is used. The acceptance

ing of the brain waves, section of parasympathetic pathways to the brain should increase hypocapnic effects

The facial nerves containing parasympathetic pathways to the pial blood vessels were exposed in cats under ether. The animals were then paralyzed with beta-erythroiden and given artificial respiration. Two minute periods of hyperventilation, which had no effect before sectioning the nerves, produced high potential, slow and spiked, activity after one or both facial nerves were cut. Stimulation of peripheral cut ends of the nerves reduced or prevented slow waves. Intravenous physostigmine in low concentration likewise prevented effects of hyperventilation, and atropine exaggerated effects previously counteracted by physostigmine. Atropine, in sufficient concentration, also produced high potential slow and spiked waves during hyperventilation without nerve section.

A parasympathetic influence on the electrical activity of the brain is demonstrated.

A cholinergic influence on cerebral metabolism and cerebral circulation is implied. A supplemental relationship between acetylcholine and carbon dioxide in homeostatic regulation of cerebral circulation is indicated.

It is suggested that a neural mechanism is indicated by which emotionally induced, autonomic changes may produce "functional" effects on brain

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achieved by a reduction of blood pressure. As long as the blood pressure continues to fall, blood flow is progressively decreased, CO_2 in the brain may remain unchanged, and there may be no change in the EEG. Only when blood pressure stops falling is CO_2 in the brain necessarily reduced and cerebral vasoconstriction properly to be assumed. And at this point increased alpha potential, not high potential slow and spiked activity, typically characterizes the EEG. This is but another of the numerous conditions in which increase of alpha potential appears symptomatic of increased cerebral vasoconstrictor tone (9). We can agree with Gibbs *et al* (17) that homeostatic vasoconstrictor regulation of blood flow is not necessarily associated with abnormality of the EEG.

If, however, during depletion of CO_2 and acetylcholine by hyperventilation there is interference with parasympathetic reimbursement of loss of acetylcholine in the brain, whether by inhibition of parasympathetic tone (11), or, as in the present experiment, by section of the nerve supply, high potential slow and spiked potentials are obtained. The possibility is suggested (1) that with reduction of the acetylcholine of synaptic transmission there may be an impairment of cerebral metabolism with associated slowing of the brain waves, and (11) that with deficiency of normal quantities of acetylcholine as vascular antispasmodic, high potential slow and spiked brain waves may be due to spasmodic effects of hypocapnia on the blood vessels. The first possibility is in harmony with evidence of the role of acetylcholine in this metabolism offered by Welsh (25) and by Nachmansohn *et al* (19, 20) and is consistent with the arterio-venous differences in oxygen and carbon dioxide reported by Gibbs, Gibbs, Lennox and Nims (17). Contradiction of the second possibility by the evidence of Gibbs *et al* that such dysrhythmias are not associated with decrease in blood flow, and by our failure to demonstrate a simultaneous decrease in brain volume is not serious. Just as it may be true that antispasmodic action of acetylcholine is essential to smooth regular, and generalized action of cerebral vasoconstrictor mechanisms, so it may also be true that no over-all change in blood flow or brain volume would accompany transient, irregular, and local constrictions or spasms of susceptible blood vessels. Concomitant, apparently generalized electrical effects conceivably might be derived under these circumstances either from the blood vessels *per se* or from associated neural mechanisms.

And finally, we would mention another possible implication of these findings—namely that there is here a mechanism by which emotionally induced autonomic changes, especially those involving inhibition of parasympathetic activity (6, 7, 12, 24) possibly exert an influence on the brain. We are provided pathways by which chronic emotional upset, especially when in association with spontaneous hyperventilation, may possibly exert an influence on both electrical activity and cerebral circulation.

SUMMARY AND CONCLUSIONS

If the inhibition of parasympathetic activity responsible for cardiac acceleration during hyperventilation is also responsible for concomitant slow-

THE ELECTROPHONIC RESPONSE TO PHASE REVERSAL

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IN 1931 Jellinek and Sheiber reported that electrical stimulation of the ear with alternating current resulted in the perception by the subject of a sound which corresponded with the frequency of the stimulus. The effect was confirmed by Fromm *et al* (3) and Stevens (9).

There are five possible explanations of this phenomenon (i) Mechanical vibrations are set up somewhere in the circuit *e.g.* at the electrode (ii) The middle ear is set into vibration and hence activates the cochlear fluid in the usual way (iii) The electrical stimulus acts directly on the cells of Corti's organ and initiates the response (iv) An electrical field is produced in the middle ear which sets up a pattern of vibration on the basilar membrane (v) The current directly stimulates the auditory nerve fibers.

In a series of experiments Gersuni and co-workers have clearly shown that the cause of the phenomenon does not lie in any artefact occurring in the circuit, such as vibrations of the electrode, or of the liquid which surrounds it, or of the skin (4, 5).

That the middle ear does not play a part in the electrophonic effect was demonstrated by the fact that sensations of tone could be elicited in the same way (i) when all the apparatus of the middle ear had been removed as in cases of disease (1) and (ii) when this mechanism was excluded by leading the current directly to the round window (3). Furthermore, immobilization of the ossicles modified the response to air-borne sounds, but the loudness and the nature of the sensations remained unaltered when electrical stimulation was employed.

Andreev and co-workers (1) stimulated with alternating currents the auditory nerve of subjects in whom the cochlea was destroyed, but the nerve was still functional. As would be expected, from the physiological properties of a nerve fiber, frequencies from 100 to 14,000 cycles per sec. were all perceived as noise, pitch was not discriminated.

These observations leave two possible explanations of the mechanism involved in pitch analysis in response to electrical stimuli (i) The current acts immediately affecting the receptor cells of the inner ear, or (ii) an electrical field is produced in the cochlea which sets up alternating mechanical forces which are similar to those produced by sound waves. The experiment now to be described was designed in an attempt to answer this question.

It is argued that if the stimulus has its action directly upon the hair-cells, a change of phase should be reproduced with considerable fidelity. The basis

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Procedure Three human subjects were employed. The external auditory meatus was cleared of wax and filled with a normal salt solution. Into the fluid was placed a short copper electrode which projected from a plaster mould fitted snugly into the opening of the meatus. An indifferent electrode consisting of a hypodermic needle was inserted subcutaneously into the neck of the subject.

When the subject reported that he heard the tone, a short period was allowed to elapse so as to familiarize him with the sound, then the phase was quickly reversed without his knowledge. Each subject was tested separately and each was instructed to report exactly what he heard, no indication of what he might expect to hear was given. Four frequencies, 600, 800, 1000 and 1200 cycles per sec. were presented to each subject and a change of phase produced at each frequency—several trials were made for each tone.

RESULTS

At all frequencies, and at each trial, the subjects experienced the same phenomenon.

S₁ reported that he heard a sudden brief period of silence followed by a "surge" of the sound as it returned.

S₂ said that he heard "a sudden stop in the sound and then it rushed back."

S₃ reported "a sudden break in the tone and then it came back louder."

In order to verify these impressions, each subject was asked to compare what he heard with a change of phase produced in a headphone. All reported that the effect was essentially the same.

DISCUSSION

When an observer listens to a steady tone whose phase is abruptly reversed, he hears a discontinuity in the sound termed by Hartbridge "the phase change beat" (6). A similar effect is produced by changing the phase of an electrical stimulus by 180°. This similarity may be taken as an indication, although not a proof, that both types of stimulation involve the same physical process in the inner ear.

The shifting of phase by 180° is mathematically equivalent to initiating, in the opposite direction, a second tone of the same frequency and double the amplitude of the original tone. Therefore, the electrophonic mechanism must include some process which is mechanically equivalent to resonance, in order that it exhibit a "phase change beat." This resonant system must be peripheral to the nerve fibers because it is impossible for them to respond as tuned elements. This suggests that a stage of transformation from electrical to mechanical forces takes place somewhere in the chain of events in the process of response to an electrical stimulus, and that the mechanical forces are less than critically damped.

Arapova and co-workers (2) exclude this stage of transformation and suggest that alternating current may immediately activate the sensory elements of the cochlea. Their rationale is that the loudness and pitch functions of electrical stimulation do not coincide with those of sound, and therefore it is improbable that the same physical events occur in response to the two types of stimuli. The flaw in their argument is that the inner ear is by no means an electrically insulated mechanism, and a certain amount of spread-

for this argument lies in the fact that the mechanism involved, whether it be the release of a chemical mediator or a physical change occurring within the cell itself, will not be characterized by anything analogous to mechanical resonance

On the other hand, if the electrical stimulus were transformed into mechanical vibrations which are less than critically damped, a phase change should be represented as a silent period. This momentary discontinuity would be a result of the force of the stimulus coming into opposition to the persisting resonant vibrations of the elements involved

METHOD

Apparatus Alternating current from a sine wave audio-oscillator (8) was fed through a simple bridge type phase changing device (see figure). This circuit employs the principle

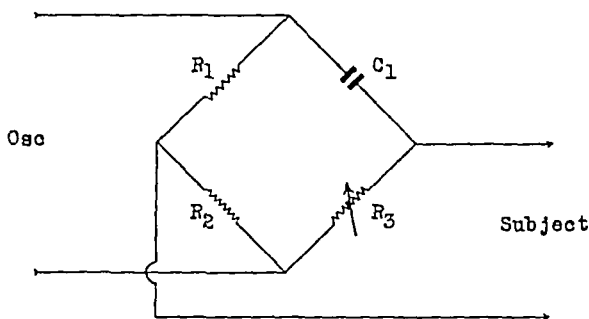


FIG 1 Circuit of phase-changing bridge. The values for the various components depend upon the characteristics of the oscillator employed

that when an A C voltage is impressed on a condenser, the maximum values of current and voltage do not occur at the same instant. The maximum voltage is reached 90° or less after the maximum current, due to the fact that a capacity tends to prevent any changes in voltage. If a resistance is placed in the circuit, the time-lag is reduced. The relationship between the time-lag and the values of capacitive reactance may be expressed,

$$\tan \theta = \frac{X_c}{R}$$

where θ is the phase angle. The phase angle is the angle through which the radius of the cycle has moved between the time of maximum current and the time of maximum voltage.

Considering the above mathematical expression, it is obvious that it is possible to vary the phase angle θ by varying the resistance R . Theoretically the range of this variation is from 0 to 90° or one-fourth of a cycle. In order to achieve a range of 0 to 180° it is necessary to employ a bridge type circuit (see figure) which is equivalent to multiplying the effect by 2.

While it is theoretically possible to vary the phase 180° using this circuit, the presence of an unavoidable amount of resistance limits the practical range to approximately 175° for the frequencies employed in this experiment. A cathode-ray oscilloscope was used to keep a constant check on the apparatus. The frequency at which the phase change was to be studied was applied to the vertical plates of the oscilloscope and the 60 cycle A C line impressed on the horizontal plates. Inspection of the aspect of the Lissagous figure presented on the screen enabled the observer to calculate the number of degrees through which the phase was shifted.

THE AFFERENT PATH OF THE PUPILLODILATOR REFLEX IN THE CAT¹

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THE CONSTANT and easily observed pupillodilatation that is evoked by afferent stimulation has led Bain, Irving and McSwiney (2), Irving, McSwiney and Suffolk (13), Harper and McSwiney (7) and McSwiney and Suffolk (18) to employ this response as a test reaction in studying the course of afferent connections from the viscera both in the peripheral nervous system and in the spinal cord. The recent observation of Ury and Gellhorn (29), Seybold and Moore (27) and Hodes (8), that this pupillodilator response in the cat occurs entirely by inhibition of oculomotor constrictor tonus (15), has suggested that it might be of equal usefulness as a test reaction in studying the distribution of the ascending reflex pathway within the more rostral portion of the neuraxis.

Because the oculomotor nucleus is the efferent structure whose activity is diminished in the production of reflex dilatation of the pupil, the entire spinal cord and brain stem below the oculomotor level remain open for the study of ascending pathways without the possibility of interference with descending pathways to spinal autonomic outflows which are involved if vasomotor (21), sudomotor (25), or nictitating membrane (1) responses are studied.

In the present investigation, therefore, pupillodilatation was evoked by stimulating the central ends of the sciatic, splanchnic and trigeminal nerves in lightly anesthetized cats, and the effect of acute section of parts of the spinal cord and brain stem upon the response was studied. In addition, the effect of destruction of parts of the brain which lie above the oculomotor level, but which might be concerned in reflex pupillodilatation (30, 32, 9, 10) was also observed.

METHODS

In cats under light nembutal or chloralosane anesthesia, the sciatic nerves were exposed in the leg and the greater splanchnic nerves through a retroperitoneal approach, shielded electrodes were applied and the nerves were crushed distally. In some experiments maxillary branches of the trigeminal nerve in the face were also stimulated. Induction shocks or 60 cycle current were employed for stimulation. Lesions of the spinal cord were made after laminectomy and lesions of the brain stem after removal of portions of the calvarium and retraction or aspiration of overlying brain parts. Lesions of the ventral portion of the medulla or upper cervical cord were made through a parapharyngeal approach. Since the ascending dilator pathway has been shown by Harper and McSwiney (7) to be

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ing with increased intensity is bound to occur. Furthermore, there is no morphological evidence for a set of electrically tuned elements anywhere in the inner ear.

It is clear, therefore, that interposed between the current stimulus and the neural response is some process which is akin to the physical events responsible for the phase change beat observed with mechanical stimulation. The implications for theories of pitch analysis and the production of cochlear microphonics are obvious. The evidence clearly contraindicates a direct action of the electrical stimulus on the cells of Corti's organ, however, until further work has been done it would be premature to designate the mechanisms involved.

SUMMARY

The response of the ear to a phase change of 180° in the electrical stimulus is found to be a brief "silent period."

Arguments are presented which favour a transduction of electrical into mechanical energy as the mechanism of the electrophonic effect.

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magnitude of the response Extension of the lesion 2 mm more medially (Fig 11b) abolished pupillary dilatation

In order to determine the excursion of the pathway within this zone, a cut was made across the ventral 2 mm of the medulla (Fig 1Ja) This did not alter the threshold but reduced the magnitude of the response After extending the lesion 2 mm dorsalward (Fig 1Jb), the pupils dilated only a trace with maximal stimulation, indicating that the majority of the pupillo-

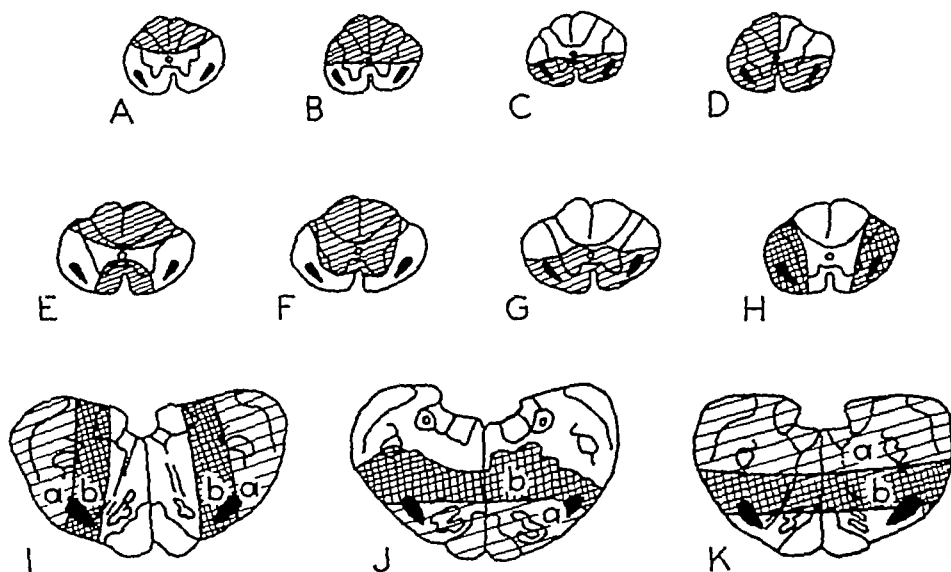


FIG 1 Outline drawings of transverse sections through the thoracic cord (A-D) cervical cord (E-H), and medulla oblongata at the obex (I-K) In these and subsequent figures the extent of lesions failing to abolish reflex pupillodilatation is indicated by oblique lining, the extent of lesions abolishing reflex dilatation is indicated by cross hatching, and the estimated position of the lateral spinothalamic tract (Papez, 20) is shown in solid black Further discussion in text

dilator fibers were interrupted Upon approaching the medulla from above, no alteration was observed in threshold or magnitude of response when the dorsal 3 mm was cut at this level (Fig 1Ka), but after extending the lesion to a depth of 5 mm (Fig 1Kb) a maximal stimulus elicited only questionable pupillodilatation

These data indicate that at the level of the caudal medulla oblongata the fibers in question pursue a course within the intermediate or lateral part of the ventral reticular formation

Sections of the medulla at the level of the restiform body At this level, section from below of the ventral 3 mm of the medulla (Fig 2Aa) elevated the threshold considerably and reduced the response to one half of the original Enlargement of the lesion to a depth of 6 mm from the base (Fig 2Ab) caused pupillodilatation to be abolished Approaching this region from the

equally crossed and uncrossed, afferent nerves were stimulated on both sides of the body and bilateral neural lesions were made at each location

After appropriate exposure the threshold stimulus for pupillary dilatation from each nerve was determined. The current strength was then increased to produce a greater dilatation which could be measured in millimeters. Selective lesions at levels of the spinal cord or brain stem were then made and the response was retested after each lesion. This was continued until pupillodilatation was abolished. Artificial respiration was employed when working in the medulla or upper cervical cord. The dilator response was observed during the first few seconds of stimulation, with pauses between stimuli, thus obviating the possibility of humoral effects.

At the end of the experiment the portion of the cord or brain studied was fixed in formahn and subsequently was sectioned serially and stained for microscopic examination. The extent of the lesion was determined by microprojection and was plotted upon outline drawings of the brain stem or spinal cord.

RESULTS

A Distribution of the ascending pupillodilator pathway

Sections of the spinal cord Attention was first given to the course of the afferent pupillodilator pathway in the upper part of the spinal cord. At the 1st thoracic level, section of the posterior columns and Lissauer's tract (Fig 1A) did not diminish pupillodilatation resulting from stimulation of the sciatic or splanchnic nerves, and interruption of either the entire dorsal (Fig 1B) or ventral half (Fig 1C) of the cord only partially reduced the response. In another instance the threshold was markedly raised and dilatation reduced to a trace following hemisection of one side and destruction of the anterolateral portion of the other (Fig 1D). The latter case indicates the importance of ascending connections for pupillodilatation in the anterolateral portion of the cord at the upper thoracic level, but since some response was still obtained after ventral destruction, this pathway is not exclusively represented there.

At the 1st cervical level, the pupillodilator response to peripheral nerve stimulation remained unaffected after section of the posterior columns and Lissauer's tract (Fig 1E), after section of the anterior columns (Fig 1E), and after evacuation of the interior of the cord by aspiration (Fig 1F), thus interrupting a considerable part of the fasciculus proprius system. A section of the entire ventral half of the cord elevated the threshold considerably and diminished the response to half the original (Fig 1G). That the ascending dilator pathway is contained entirely within the lateral columns at the 1st cervical level, is seen from the fact that bilateral sections of the lateral columns alone (Fig 1H) in two instances caused complete abolition of the dilator response with even the most intense peripheral stimulation (see also 7).

In the upper cervical cord, therefore, the afferent pathway evoking pupillodilatation ascends diffusely in the lateral columns.

Sections of the medulla at the level of the obex At the level of the caudal portion of the medulla oblongata the lateral extent of the dilator pathway was first determined. Bilateral section of the lateral 4 mm of the medulla (Fig 1Ia) produced only a slight elevation in threshold, with no change in the

A section extending from the floor of the ventricle to the base of the pons, and 2 mm to each side of the midline (Fig 3Aa) did not alter the pupillary threshold or response to sciatic or splanchnic stimulation, however, extension of the lesion 2 mm more laterally but only to a depth of 3 mm (Fig. 3Ab) abolished all response to maximal stimulation. In another animal bilateral cuts were made from 2 to 4 mm from the midline and 4 mm deep

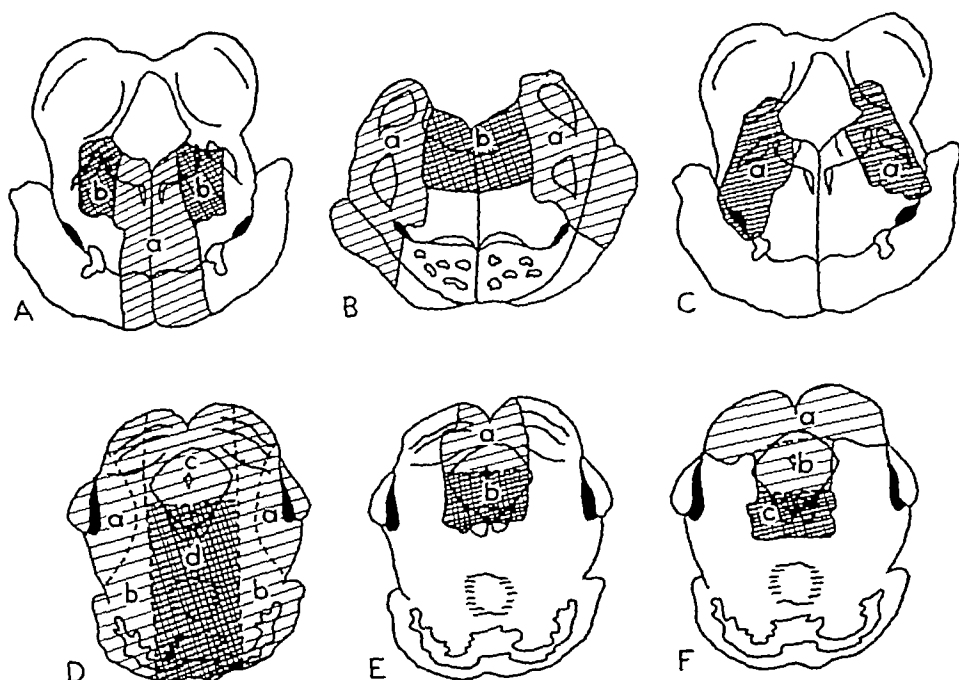


FIG 3A-F Outline drawings of transverse sections through the pons and midbrain
Lesions indicated as in Fig 1 Further discussion in text

(Fig 3Ca), after these lesions no alteration in pupillary size resulted from afferent stimulation

From these results it is seen that the dorsal trend of the pupillo dilator pathway, begun more caudally, continues so that at the pontile level the pathway occupies a position in the dorsal reticular formation close beneath the grey matter around the central canal. Further examination reveals that the pathway has also commenced to shift medially, not to a midline distribution but rather to a paramedian position. Here, as more caudally, the distribution of the afferent dilator pathway is seen to be definitely removed from that of the lateral spinothalamic tract

Sections of the midbrain Lesions at this level were made in a plane passing through the caudal part of the main oculomotor nucleus only a short distance behind the Edinger-Westphal component, a reduction in the constrictor activity of which yielded pupillo dilator in these experiments. Data

dorsal side, the threshold was raised slightly and the response decreased following a lesion 2 mm deep (Fig 2Ba) across the dorsal portion of the medulla. When this section was extended to a depth of 4 mm (Fig 2Bb) only a barely perceptible increase in pupillary diameter resulted from maximal stimulation.

To ascertain the lateral extent of the ascending pathway, destruction of the region delimited above was extended progressively from the midline. Section of the medulla to a depth of 3 mm, and 4 mm from the midline on either side (Fig 2Ca) had no influence on pupillodilatation, whereas continuation of the lesion 2 mm more laterally (Fig 2Cb) abolished all response to maximal stimuli. In two other animals it was similarly observed that pu-

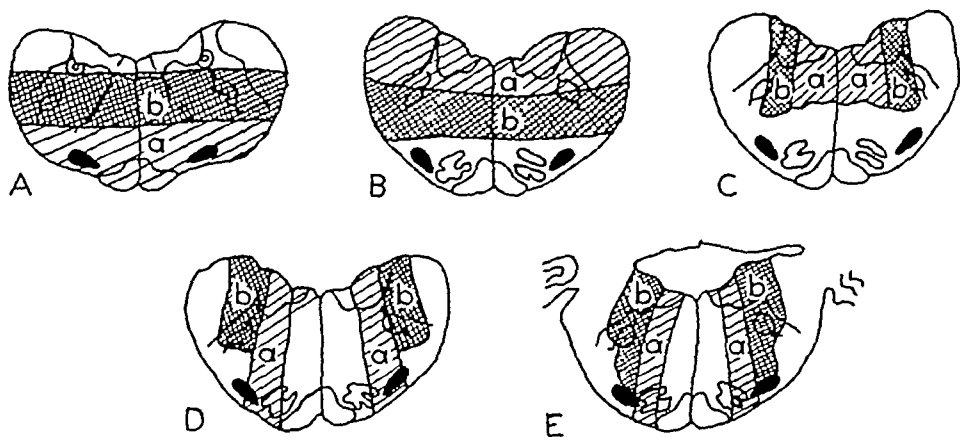


FIG 2A-E Outline drawings of transverse sections through the medulla oblongata at the level of the restiform body. Lesions indicated as in Fig 1. Further discussion in text.

pillary dilatation was abolished after bilateral destruction of that portion of the lateral reticular formation between the vestibular nuclei and the nucleus of the spinal fifth tract (Fig 2Db, Eb).

An examination of the results obtained after lesions at the level of the restiform body indicates that the ascending dilator pathway still occupies a position here in the intermediate or lateral part of the bulbar reticular formation. The pathway has, however, commenced to shift dorsalward and is definitely more dorsally situated than was the case at the level of the obex. As a result of this dorsal migration, the distribution of the afferent pathway evoking pupillodilatation is seen to be distinct here from that of the lateral spinohthalmic tract.

Sections at the level of the pons. Lesions destroying the lateral portions of the pontile brain stem at the anterior end of the fourth ventricle (Fig 3Ba) did not alter the threshold or magnitude of the pupillary response. Interruption of the remaining medial part of the brain stem to a depth of 3 mm (Fig 3Bb) abolished all reaction to maximal stimulation.

exerted directly at the level of the midbrain, but the possibility remains that the pathway continues to some higher level, the participation of which is essential for the response. An attempt was, therefore, made to determine whether this was the case.

In several acute experiments the cerebral cortex was extirpated and the

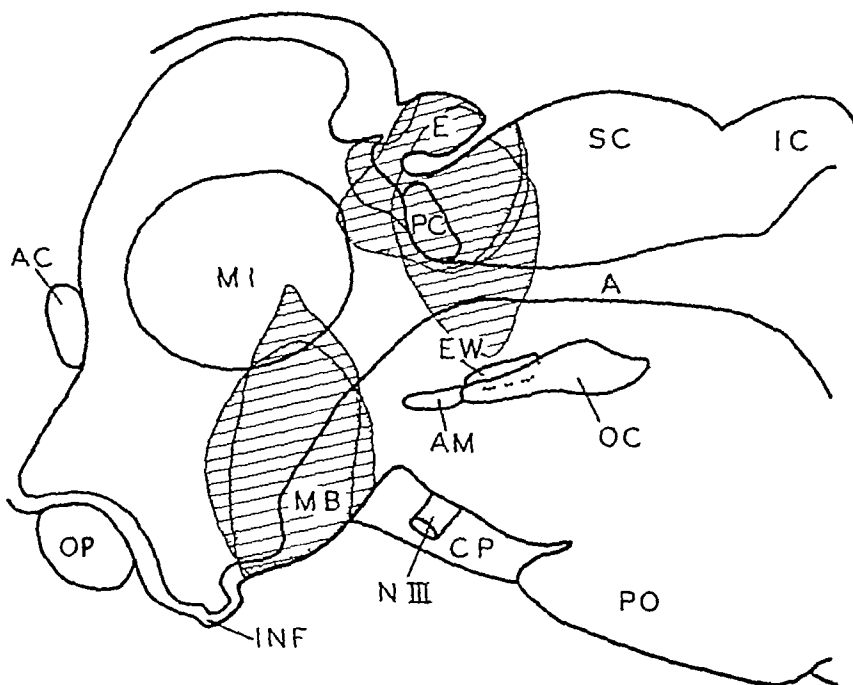


FIG 4 Reconstruction of the midsagittal plane of the rostral brain stem of the cat, upon which is projected the extent of lesions in the hypothalamus and pretectal region. Further discussion in text. Abbreviations are as follows:

A —aqueduct	MB —mammillary body
AC —anterior commissure	MI —massa intermedia
AM—anteromedian part of oculomotor nucleus	N III—oculomotor nerve
CP —basis pedunculi	OC —main oculomotor nucleus
E —pineal body	OP —optic chiasma
EW—Edinger-Westphal nucleus	PC —posterior commissure
IC —inferior colliculus	PO —pons
	SC —superior colliculus

diencephalon removed from before backward, the threshold and magnitude of the dilator response to sciatic nerve stimulation being tested at intervals. Impairment in the response did not occur until the caudal portion of the diencephalon was removed, and then appeared to result more from involvement of the transition from dorsal thalamus to tectum than from injury to the hypothalamic region.

Nevertheless the effect upon the dilator response to afferent stimulation

here were gathered, therefore, in as close a proximity to the Edinger-Westphal nucleus as was possible without injuring it. That this nucleus was not injured in any of the experiments was seen both from the microscopic examination of the sections, and from the fact that the pupils remained narrow after all of the lesions, instead of dilating widely as occurs when this nucleus is damaged.

In one animal the region of distribution of the lateral spinothalamic tract was interrupted bilaterally (Fig 3Da). This was not followed by any alteration in the pupillodilator response, nor did any occur when the entire lateral midbrain was sectioned on either side (Fig 3Db). There was still no impairment following section of the remaining medial portion to below the cerebral aqueduct (Fig 3Dc), but abolition of the dilator response followed interruption of the remaining ventromedial area (Fig 3Dd).

In another instance section of the medial portion of the midbrain down to the level of the aqueduct (Fig 3Ea) did not alter the threshold or magnitude of the pupillodilator response to peripheral nerve stimulation. When, however, this cut was extended ventrally to interrupt the remaining central grey and the immediately adjacent portion of the midbrain tegmentum (Fig 3Eb), the dilator response was abolished. Similar results were encountered in another animal, in which the dilator response was unaltered after removing the entire tectum of the superior colliculus, but leaving the pretectal region intact (Fig 3Fa), or after interruption of all but the most ventral part of the aqueductal grey (Fig 3Fb). Interruption of the remainder of the ventral central grey and the tegmentum immediately adjacent to it (Fig 3Fc), as before, abolished reflex pupillodilatation.

The results following sections in the midbrain, like those at lower levels, indicate that the distribution of the afferent pathway evoking pupillodilatation is not coincident with that of the lateral spinothalamic tract. The fact that the dilator response remained unaffected after removal of the superior colliculi demonstrates also that connections to or from the tectum are not concerned in its mediation. The dorsal and medial shift of the ascending dilator pathway, encountered at lower levels, continues and increases so that the pathway is directed forward through the midbrain close to the midline, either in the most ventral part of the central grey of the aqueduct or in the tegmentum immediately beneath it.

The significance of the shifting course of the afferent dilator pathway through the brain stem now seems evident, for its end result is to deliver this constrictor-inhibitory pathway either exactly to the oculomotor nucleus or to its close vicinity. This observation suggests that the pathway may terminate there and exert its inhibitory action upon the oculomotor nucleus directly, without passage to or mediation through some higher level of the brain.

B The effect of lesions above the midbrain

The convergence of the ascending pupillodilator pathway upon the region of the oculomotor nucleus suggests that its constrictor inhibitory action is

Other autonomic reflex arcs have been found either to traverse levels of the central nervous system above their motor outflow, or to require the influence of such parts for the initiation of the response. The galvanic skin reflex to "psychic" stimuli is abolished after ablation of the cerebral cortex of the cat (25). The reflex retraction of the nictitating membrane is altered after cortical extirpation and is abolished by pontile transection of the brain stem (19). The peripheral vasomotor response to painful cutaneous stimulation in man involves neural structures lying rostral to the medulla oblongata (17). Peripheral vasoconstriction to cold, on the other hand, can occur as a spinal reflex (24).

It may be surprising that reflex oculomotor inhibition, whose terminal effect occurs at a level as far rostral as the midbrain, does not involve the participation of potential, supranuclear, oculomotor inhibitory mechanisms known to be present in the closely adjacent hypothalamus (9) or in the cerebral cortex (10). But with the possible exception of the pretectal region, destruction of which alters oculomotor tonus, the pupillodilator reflex is not impaired by the extirpation of neural structures above the level of the oculomotor nucleus where its constrictor inhibitory action is effected.

The second aspect of these results that merits discussion concerns the distinctness of distribution within the central nervous system of the afferent connections which inhibit oculomotor activity, and those afferents that form the lateral spinothalamic tracts, which, in man at least, subserve the sensation of pain (11, 12, 23, 26, 31). It has previously been found that the intramedullary pathways for some autonomic and other reflexes, thought to be evoked by nociceptive stimuli, have a course distinct from that of the lateral spinothalamic tract within the spinal cord.

Thus the pressor reflex to sciatic stimulation is dependent upon afferent connections coursing apparently for the length of the cord in the tract of Lissauer (21). Vasomotor and other reflexes caused by stimulating the sympathetic chain, or by dilating the cystic and biliary ducts, appear to be initiated by intramedullary afferents travelling in propriospinal systems (4, 5).

Afferent pathways for other autonomic reflexes have commonly been found to travel upward in the lateral funiculus of the cord, where the possibility of their identity with the lateral spinothalamic tract, present there, is more likely, but is not necessarily proven. Among such afferents are those for the depressor response to sciatic stimulation (21), peripheral vasoconstriction to painful cutaneous stimulation (17), retraction of the nictitating membrane (1), and pupillodilatation (7, present experiments) evoked by afferent nerve stimulation.

In the case of the two latter reflexes the afferent pathway is approximately equally crossed and uncrossed, a feature differing from the crossed distribution of the lateral spinothalamic tract. Furthermore, in the case of the pupillodilator reflex some afferent connections still remain intact after ventral hemisection of the cord, with bilateral interruption of the lateral spinothalamic tracts (7, Fig. 1C and G of the present study). The present

of lesions destroying the entire hypothalamus at the mammillary level was studied in two animals (Fig 4) These were chronic preparations, the sciatic nerve being stimulated under chloralosane one month after hypothalamic destruction A threshold dilator response was elicited with minimal current, and with stronger stimulation marked pupillodilatation was obtained without difficulty Such reflex dilatation appears, therefore, to be independent of the hypothalamus (see also 32)

To investigate the other neural region above the oculomotor level, which acute experiments indicated might be involved in reflex pupillodilatation, large lesions destroying the pretectal region and adjacent parts of the diencephalon and midbrain were made in three animals (Fig 4) After a 2-week interval, the sciatic nerve was stimulated under chloralosane

The pupils were widely dilated (10–11 mm) in these animals and the constrictor reaction to light was abolished Because of the extreme width of the pupils, eserine was instilled into the conjunctival sacs and time was allowed for the pupils to become constricted before nerve stimulation was begun In one case sciatic stimulation with minimal current (0.2 V) gave perceptible dilatation In the other animals, stimulation with 0.5 V gave a dilatation of 1–2 mm In each animal, further increase of the intensity of stimulation to as high as 12 V did not result in any further increase in the width of the pupils The effect of pretectal destruction upon reflex pupillodilatation was, therefore, greatly to diminish the magnitude of the response, while leaving its threshold low and within normal limits

This observation does not necessarily indicate that a part of the afferent dilator pathway traverses the pretectal region, but rather its explanation appears to us to lie in the dominant role of the pretectal area in maintaining excitation in the constrictor part of the oculomotor nucleus (16) In the absence of the pretectal region, constrictor activity is almost in abeyance, as is evident from the extreme width of the pupils, and only a residue of excitement remains to be inhibited by afferent stimulation

DISCUSSION

The first aspect of the results just presented that requires comment concerns the neural level at which oculomotor inhibitory action is effected by afferent nerve stimulation There is every indication that this occurs at the rostral end of the midbrain, at the level of the preganglionic outflow whose diminished activity yielded pupillodilatation Supranuclear structures lying above the midbrain did not appear to play any essential role in the response

This is not an unprecedented finding for Brooks (3) has shown that rises in blood sugar, increments in arterial pressure and heart rate, and contraction of the denervated nictitating membrane can be reflexly induced from the spinal cord below C6 Some of these responses, however, were, at the most, less than half as great as those obtained with more rostral brain parts intact, and Brooks concludes that the centers chiefly responsible for normal reflex excitation of the sympathicoadrenal system are located in the medulla or some higher region of the brain

Mention of the peripheral nerves raises a third point to which attention could be called—that of central connections made by afferent fibers in visceral and somatic nerves. In these experiments the effect of lesions on pupillodilatation evoked by sciatic and by splanchnic nerve stimulation was routinely compared and in no instance were the dilator responses from these two sources ever dissociated, in every case they remained unaffected or were paired or abolished together (see also 7). These results suggest that both somatic and visceral sources contribute to a common secondary pathway.

A few comparisons of the effect of trigeminal nerve stimulation may also be mentioned. In two animals with pontile lesions abolishing the dilator response to sciatic and splanchnic stimulation, and sparing primary trigeminal connections (Fig. 3A and C), pupillodilatation evoked from the trigeminal nerve was reduced to a trace. In two animals with midbrain lesions (Fig. 3E and F) abolishing dilatation from sciatic and splanchnic stimulation, the dilator response from trigeminal excitation was also abolished. These observations suggest that the common spinal pathway conveying afferent impulses from somatic and visceral sources receives trigeminal contributions in its passage forward through the brain stem.

The general conclusion to which the present results point is that pupillodilatation and pain evoked by stimulating afferent nerves are mediated by equal and separate intramedullary connections. These results do not favor the view that this dilator effect is mediated by diencephalic or other collaterals from the afferent pain pathway, both because of the dichotomy of the afferent connections concerned, and because the diencephalon and higher regions of the brain do not seem to be involved in the response. Whether afferent stimulation of a painful nature could provoke pupillodilatation in the chronic absence of the reflex pathway here outlined, by some more devious route, or through diencephalic or cortical excitation, is uncertain, it has not done so in these acute experiments under anesthesia. At present only a coincidental relation between pupillodilatation and pain would seem to exist, and use of the pupillodilator effect as an indicator of either somatic or visceral pain would appear to be open to question.

Whether the significance of the intramedullary pathway here outlined is limited to its role in evoking pupillodilatation upon afferent stimulation, or whether it has the more general function of bringing into play some of the additional autonomic and somatic responses that are usually elicited in such circumstances, still remains to be determined.

SUMMARY

The pupillodilatation resulting from sciatic, splanchnic and in some instances trigeminal nerve stimulation has been observed after various lesions of the spinal cord and brain in lightly anesthetized cats.

This response is known to result from inhibition of oculomotor constrictor activity, the present study adds the observation that destruction of parts of the brain above the oculomotor nucleus does not impair the effect, which is evidently completed at the midbrain level.

experiments add the observation that dorsal hemisection of the cord (Fig 1B), which should not impair conduction in the lateral spinothalamic tracts, and which according to one view (6) should facilitate it, nonetheless reduces the pupillodilator reflex. In addition, Karplus and Kreidl (14) have demonstrated that the feline pupillodilator response to sciatic stimulation is still preserved after a lateral hemisection of one side of the cord at the thoracic level and a second hemisection of the opposite side at the cervical level. The ascending connections concerned cannot be made up solely of long pathways in the white matter of one or the other sides, but must involve crossed relays.

There is no compelling evidence, therefore, that afferent connections for autonomic responses, which ascend in the lateral funiculus of the spinal cord, are identical with the lateral spinothalamic tract, and there are some definite indications that dual or multiple afferent pathways may be present, with features independent of one another.

Little has been known concerning the more rostral distribution in the brain stem of afferent pathways for autonomic responses which require this or higher levels of the brain for their completion. Marquis and Williams (17) report three patients with unilateral bulbar or pontile lesions destroying the lateral spinothalamic tract, in whom there was a reduced vasoconstrictor response to painful or thermal stimuli on the hypalgæsic side of the body, which fact suggests that the lateral spinothalamic tract is the ascending brain stem pathway for this vasomotor reflex. Disturbances in patients with similar lesions have been explained by Stead *et al* (28), on the other hand, as due to the interruption of descending brain stem tracts concerned with various functions of the autonomic system.

Unless the course of the lateral spinothalamic tract in the cat differs greatly from that in other animals and man, the present study has demonstrated an ascending pathway evoking oculomotor inhibition whose distribution is quite separate from that of the pain pathway at bulbar, pontile and midbrain levels. This ascending pupillodilator path passes obliquely forward through the bulbar reticular formation to gain a paramedian position in the dorsal pontile tegmentum, and ascends through the midbrain in or closely adjacent to the ventral central grey of the aqueduct. Its distribution does not appear to correspond to any hitherto described ascending pathway, and its precise structural identity remains unknown. This is not surprising, for through much of its ascending course this pathway traverses the *terra incognita* of the brain stem reticular formation.

There is a possibility that the oculomotor inhibitory and pain afferents are also distinct from one another in the peripheral nerves. The first-order neurons of the lateral spinothalamic tract are generally believed to be the small cells of the dorsal root ganglia whose peripheral processes constitute the unmyelinated and fine myelinated fibers in the peripheral nerves (22). These small fibers exhibit a high threshold to direct electrical stimulation, but the thresholds of both the sciatic and splanchnic nerves to stimuli evoking pupillodilatation in the present experiments were exceedingly low.

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A common intramedullary pathway conveys afferent impulses from each of the sources studied to the midbrain. This pathway ascends through the lateral funiculus of the spinal cord, traverses the reticular formation of the medulla, gains a paramedian position in the dorsal pontile tegmentum, and ascends through the midbrain in or near the ventral central grey of the aqueduct.

This oculomotor inhibitory pathway seems to be distinct from the lateral spinothalamic tract throughout its course and pupillodilation and pain evoked by stimulating afferent nerves would appear to bear only a coincidental relation to one another.

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CHANGES OF WEIGHT AND NEUROMUSCULAR TRANSMISSION IN MUSCLES OF IMMOBILIZED JOINTS*

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(Received for publication, May 18, 1944)

THOMSEN, ALTAMIRANO and LUCO (4) demonstrated that synaptic changes occur in a muscle whose tendon has been cut 3 to 15 days previously, and suggested the possibility that section of the tendon would result in a great exaggeration of the postural reflexes, since some of the muscles pulling on the joint now lack antagonists. More recently, it has been found that these synaptic changes disappear when the tendon spontaneously regrows, re-establishing the equilibrium at the joint despite the persistence of muscular atrophy produced by tenotomy (5).

In order to determine the effect of position of the limb and of stretch of the muscle on neuromuscular transmission, studies were made in animals in which the tibio-tarsal joint had been immobilized by a plaster cast in one of three positions, namely, hyperflexion, hyperextension, and intermediate. The responses of the soleus muscle to different frequencies of stimulation were recorded, and the sensitivity of this muscle and of the tibialis anticus to curare was determined. In each experiment the homologous muscle of the opposite leg was used as a control. Hyperflexion produced maximal stretch of the soleus and maximal shortening of the tibialis, whereas hyperextension produced the opposite. In addition to the neuromuscular transmission, the weight of the two muscles, thus exposed to high or low tension, was studied.

METHOD

In cats anesthetized with ether, bandages impregnated with plaster were applied directly over the skin from the metatarsals to the knee. Occasionally edema or small decubitus ulcers were formed. Four to 30 days later an acute experiment was performed under Nembutal (Abbott), in a dose of 0.033 g per kg of body weight dissolved in 1 cc of 25 per cent urethane. A cannula was inserted in the trachea in order to apply artificial respiration when necessary. The tibia was fixed by drills and the tendon was attached to the short arm of a lever, pulling on rubber bands. The amplification of the lever was 6 to 8 times. Silver electrodes were applied to the sciatic nerve, which had been isolated and sectioned above the hip. The nerve was stimulated by condenser discharges controlled electronically. Curare (Merck) was injected intravenously. The muscles of experiments performed after 9 to 16 days of immobilization were carefully dissected and weighed immediately.

RESULTS

1 *Soleus muscle stimulated indirectly at various frequencies.* A *Hyperextension.* In the 8 animals studied the response to indirect stimulation at frequencies from 128 to 300 per sec. was characterized by a greater development of the third stage of neuromuscular transmission as compared to the

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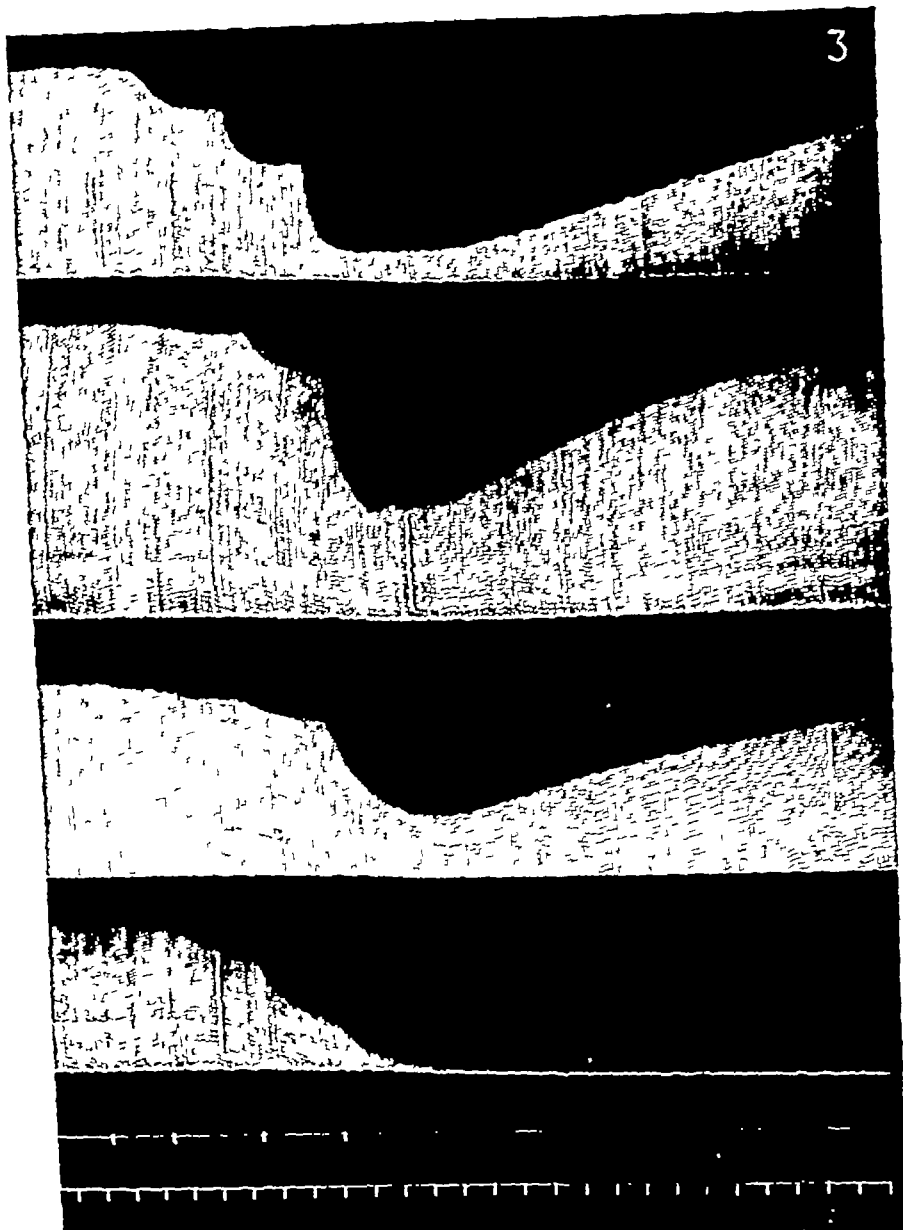


FIG 3 Effects of curare after immobilization of the tibio-tarsal joint in hyperflexion for 12 days Top line normal tibialis anticus 2nd line immobilized tibialis 3rd line immobilized soleus 4th line normal soleus Frequency of stimulation of sciatic nerve 1 per 4 sec Upper signal injection of doses of curare, after the fourth signal artificial respiration was begun Time 1 min

exceptions, since on comparing the per cent change in the muscles of the immobilized leg it is seen that algebraically they follow the same law

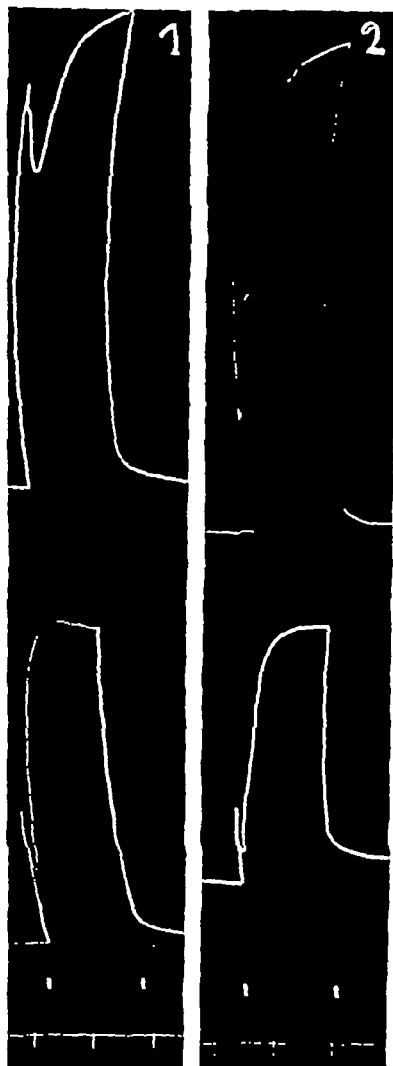


FIG 1 Stages of neuromuscular transmission of soleus muscle after immobilization of tibio-tarsal joint in hyperflexion. Upper line normal. Lower line after 7 days of immobilization. Frequency of stimulation of sciatic nerve, 200 per sec. Time 1 min.

FIG 2 Stages of neuromuscular transmission of soleus muscle after immobilization of tibio-tarsal joint in hyperextension. Upper line normal. Lower line after 12 days of immobilization. Frequency of stimulation of the sciatic nerve, 200 per sec. Time 1 min.

first, than occurs in the control (Fig 2). These stages, as seen in the normal cat, are described by Rosenblueth and Cannon (2).

B Intermediate position In 3 animals the results were the same as those found in hyperextension.

C Hyperflexion Fifteen experiments were performed, in 10 of which the third stage was greater in relation to the first than in the control (Fig 1). In the other 5 experiments the results were different. In some, the third stage was greater in relation to the first in the control than in the immobilized limb, when tested at 128 per sec, whereas at 200 or 300 per sec the responses of the two muscles were the same. In others, the greater development of the third stage in the control than in the immobilized limb was evident at all the frequencies studied.

2 Effects of curare in soleus and tibialis anticus In all the positions investigated and in all experiments performed the muscles of immobilized limbs were found to be more resistant to the effects of curare than were those of control limbs (Fig 3 and 4).

3 Weight of the muscles On comparing the weights of the soleus or tibialis anticus muscles of the two limbs of normal animals, the per cent difference was found to be 4.8 ± 1.1 (Table 1).

A Hyperflexion In this position the soleus had been in a state of maximal stretch, its weight was greater than that of the control. The tibialis had been maximally shortened, and its weight was less than that of the control. These results and the average changes in weight are presented in Table 2.

B Hyperextension Inversely, when the soleus had been maximally shortened, its weight decreased, whereas the weight of the maximally stretched tibialis increased (Table 3).

In 3 instances there were exceptions to this rule. These are, however, only apparent

Table 2 *Tibio-tarsal joint fixed in hyperflexion*

Days of immobilization	W of soleus (g)		Per cent difference	W of tibialis (g)		Per cent difference
	N	I		N	I	
10	3 050	2 870	- 5 9	5 600	4 550	-18 7
12	2 630	2 200	-16 3	4 840	3 260	-32 6
10	1 510	1 670	+10 5	2 430	2 345	- 3 4
10	1 620	2 390	+47 5	2 130	2 040	- 4 2
11	1 620	1 850	+14 1	3 930	3 300	-16 0
11	1 400	1 780	+27 1	2 550	2 090	-18 0
10	2 480	2 860	+15 3	3 950	3 640	- 7 8
10	2 300	2 495	+ 8 4	3 640	3 400	- 6 5
14	1 525	1 940	+27 2	2 075	1 660	-20 0

N normal muscle I immobilized muscle

Average per cent increase of weight of soleus, $+14.2 \pm 6.2$

Significant difference from normal muscles, 1.49

Average per cent decrease of weight of tibialis, -14.1 ± 3.1 per cent

Significant difference, 2.73

Note the low significant difference of the soleus is due to the opposite sign of the first 2 experiments. The average per cent increase of the remaining 7 experiments is 21.4 ± 5.1 per cent and the significant difference is 3.18

does tenotomy of this muscle. This result appeared in the series of experiments involving fixation in hyperextension and the intermediate position, and in most of the series in hyperflexion.

Section of the Achilles tendon produces a certain position of the tibio-tarsal joint and a shortening of the soleus muscle. This position is the same as that of the joints immobilized in hyperflexion, but the tension of the muscles is different. In contrast, hyperextension produces tension of the soleus similar to that found in tenotomy but a different position of the joint. The differ-

Table 3 *Tibio-tarsal joint fixed in hyperextension*

Days of immobilization	W of soleus (g)		Per cent difference	W of tibialis (g)		Per cent difference
	N	I		N	I	
10	2 175	1 360	-37 4	4 810	5 280	+ 9 7
10	1 945	1 420	-26 9	3 930	4 310	+ 9 6
15	1 200	0 750	-37 5	3 260	3 120	- 4 2
16	1 710	0 840	-50 8	3 090	3 930	+27 1
10	2 300	1 170	-49 1	4 380	5 710	+30 3
9	1 845	1 490	-19 2	3 315	4 010	+20 9
12	1 935	0 860	-55 5	3 290	4 435	+34 8
10	2 150	1 435	-33 2	5 020	6 580	+31 0
13	2 100	1 090	-48 0	3 060	4 750	+55 2

N normal muscle I immobilized muscle

Average per cent decrease of weight of soleus, -39.7 ± 4 per cent

Significant difference, 8.41

Average per cent increase of weight of tibialis, $+23.8 \pm 5.3$ per cent

Significant difference, 3.48

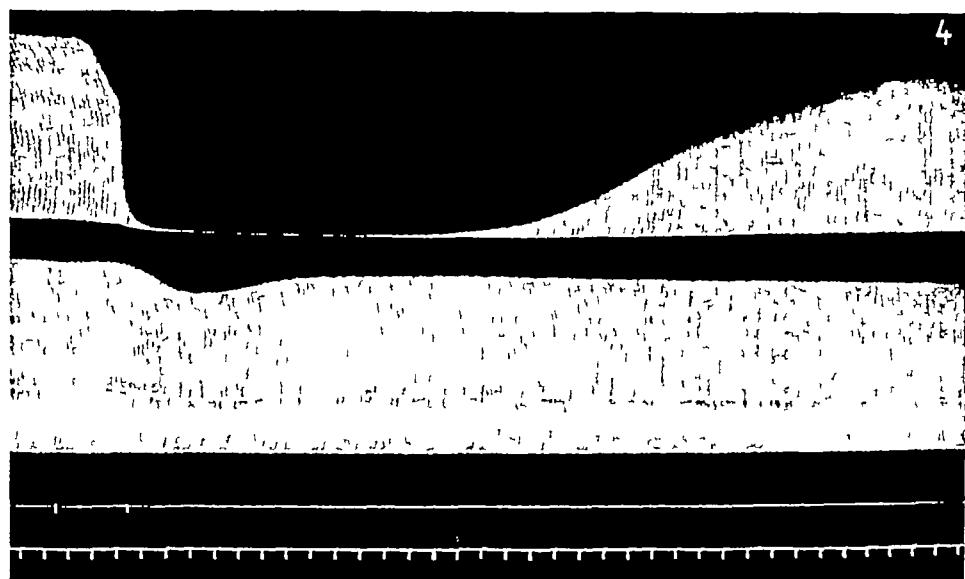


FIG 4 Effects of curare after immobilization of the tibio-tarsal joint in hyperextension for 15 days Tibialis anticus Above normal Below immobilized Frequency of stimulation of the sciatic nerve 1 per 3 sec Upper signal injection of doses of curare, after the second signal artificial respiration was begun Time 1 min

DISCUSSION

1 *Synaptic effects* Fixation of the tibio-tarsal joint changes the responses of the soleus muscle stimulated at different frequencies in the same way as

Table 1 Weight of normal muscles in grams

Muscle	R	L	Per cent difference
Soleus	1 900	2 010	5 7
Soleus	1 690	1 480	14 2
Soleus	1 770	1 765	0 3
Soleus	1 920	2 080	8 3
Soleus	1 900	1 785	6 4
Soleus	2 490	2 710	8 8
Soleus	2 500	2 445	2 2
Tibialis	3 395	3 330	1 9
Tibialis	2 090	1 900	10 0
Tibialis	3 610	3 690	2 2
Tibialis	2 740	2 680	2 3
Tibialis	5 020	4 890	2 6
Tibialis	5 610	5 770	2 8
Tibialis	4 650	4 640	0 2

Average per cent difference between normal muscles, 4.8 ± 1.1 per cent

tension in each case produced an increase of weight, whereas a decrease of tension produced a decrease of weight

Most authors assign edema, ischemia, or damage due to compression as the most important factor in causing the atrophy of immobilization. The results reported here indicate that these factors are not important

SUMMARY

1 The influence of fixation of the tibio-tarsal joint on the neuromuscular synapse and the weight of the soleus and tibialis anticus muscles was studied in cats anesthetized with Nembutal. The joint was fixed in hyperflexion, hyperextension and intermediate between these two

2 After immobilization in these 3 positions, these muscles show the same changes in neuromuscular transmission as are found in tenotomized muscles. At high frequencies of stimulation there was a greater development of the third stage of neuromuscular fatigue in relation to the first stage than occurred in the control muscles (Fig. 1 and 2)

3 The immobilized muscles resemble tenotomized muscles also in being less sensitive to curare (Fig. 3 and 4)

4 Fixation in hyperflexion for up to 14 days causes an increase in the weight of the soleus and a decrease in the weight of the tibialis (Table 2)

5 Conversely, fixation in hyperextension causes a decrease in the weight of the soleus and an increase in the weight of the tibialis (Table 3)

6 In the discussion the hypothesis is presented that in immobilization, as in tenotomy, the abnormal tension to which the muscle is exposed causes the neuromuscular changes by a reflex mechanism

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ent results of some of the experiments in hyperflexion may be explained if these two factors are assumed to act in causing the synaptic abnormality seen at the frequencies studied, and if the tension of the muscle is the more important of the two. These factors would act by a reflex mechanism.

No matter what position the joint was fixed in, the immobilized soleus was more resistant to curare than the control. The same results hold for the tibialis, in which, however, we did not study the responses to various frequencies because we are not familiar with the responses of normal muscles.

It might be argued that immobilization in the intermediate position could produce no change of neuromuscular transmission. But there are two objections to this view: (1) it is impossible to know exactly what position is intermediate, so that the soleus is neither stretched nor shortened, and (2) fixation itself may cause synaptic changes. The latter explanation seems unsatisfactory since tenotomized muscles present the same changes, yet in this case we cannot properly speak of fixation.

If there should be a certain relation between the quantity of muscular mass and of nerve, and perhaps also of mediator produced during synaptic transmission, this proportion should be altered by a change of weight of the muscle. Now if the change in sensitivity to curare described above were explainable by an alteration in this proportion, one would expect in one series of experiments an increase of sensitivity, and in the other set a decrease. But, as noted above, only a decrease of sensitivity to curare occurs, regardless of the direction of the change of muscle mass. The same arguments may be applied in the case of the changes in the responses to different frequencies.

In summary, the synaptic changes induced by tenotomy are the same as those found after fixation of the joint.

Probably the most important factor in all these conditions is the abnormality, either increase or decrease, of the tension or degree of stretch to which the muscle has been exposed.

2 Weight of the muscles. The soleus and tibialis anticus muscles were chosen for this determination for the following reasons: (1) they are well known from the point of view of synaptic function, (2) they are easy to dissect and hence may be weighed exactly, and (3) since they are antagonists, immobilization in one extreme position produces high tension in one and low tension in the other.

A review of the literature on immobilization and muscular atrophy reveals contradictions in the experimental results. Thus Lippmann and Selig (1) showed that in the rabbit long periods of immobilization produced no atrophy. On the other hand, other investigators (for references see Solandt, Partridge and Hunter, 3) found atrophy. In view of the results reported here, these differences may have been due to differences of the position in which the joint was fixed.

In regard to this relation between the position of fixation of the joint and the resulting changes in muscular weight, the fundamental factor would seem to be the tension to which the muscle had been exposed. Thus an increase of

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RELATION OF CEREBRAL CORTEX TO SPASTICITY AND FLACCIDITY*

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(Received for publication June 23, 1944)

SINCE THE TIME of Hughlings Jackson the subject of flaccid and spastic paralysis has received wide attention. Although in recent years the association of spasticity‡ with extrapyramidal lesions and flaccidity with the pyramidal (2, 5, 9, 10, 13, 14) has been established, questions remain unanswered which have been recently reconsidered in the light of new therapeutic neurosurgical procedures for the symptomatic relief of spasticity (1, 11).

Certain preliminary observations on spasticity and flaccidity following removal of various areas of the sensorimotor cortex in monkeys led to the present investigation. These were (i) although removal of area 6 caused spastic paresis and removal of area 4, flaccidity, simultaneous removal of both areas produced greater spasticity than did excision of area 6 alone, (ii) primary removal of the postcentral gyrus caused flaccidity with minimal paresis; (iii) removal of an entire hemisphere caused more marked spasticity and paresis than did removal of the motor and premotor areas alone.

Previous investigators have already thoroughly explored the sensorimotor areas rostral to the central sulcus in monkeys and chimpanzees in order to determine the effect of their removal on motor performance. Fulton has shown in a number of primates (2, 4), as has Hines in *Macaca mulatta* (6) that excision of the excitable motor area, area 4, gives rise to contralateral hemiparesis with little or no spasticity. Similarly, if the principal descending projection of this area, the corticospinal tract, is cut at the level of the medullary pyramids in cats (9, 10, 13) or monkeys (14) no spasticity is evident.

It has also been established that primary injury to area 6 results in reflex grasping and slight increase of resistance to passive flexion and extension (4, 5). If area 4s, lying between areas 4 and 6, is destroyed, alone or in combination with area 4 or area 6, spasticity results (6). Furthermore, lesions to area 6 intensify ipsilateral spasticity and bilateral removal of areas 4 and 6 results in great paresis and spasticity in all extremities (2).

There has been less thorough analysis of the large pyramidal and extra-

* Aided by a grant from the Fluid Research Fund, Yale University School of Medicine.

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‡ The term "spasticity" as used herein refers to any reflex increase in resistance to passive movement, no attempt has been made to appraise the quality of the resistance or to distinguish the rigidities, so called, from the classical spasticities in which lengthening and shortening reactions can be demonstrated.

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- The materials of this helpful, widely used book are derived out of everyday contacts with pediatricians, consultation work in a large pediatric clinic and dispensary, a long time collaboration with private practitioners and with the diverse daily problems of the child caring agencies of a metropolitan community (its schools, orphan ages, hospitals, welfare and community organizations, courts, custodial and correctional institutions), and of teaching activities at Johns Hopkins University School of Medicine

WIDELY USED BY PEDIATRICIANS, CONSULTATION WORK IN A LARGE PEDIATRIC CLINIC AND DISPENSARY, A LONG TIME COLLABORATION WITH PRIVATE PRACTITIONERS AND WITH THE DIVERSE DAILY PROBLEMS OF THE CHILD CARING AGENCIES OF A METROPOLITAN COMMUNITY (ITS SCHOOLS, ORPHAN AGES, HOSPITALS, WELFARE AND COMMUNITY ORGANIZATIONS, COURTS, CUSTODIAL AND CORRECTIONAL INSTITUTIONS), AND OF TEACHING ACTIVITIES AT JOHNS HOPKINS UNIVERSITY SCHOOL OF MEDICINE

A reliable guide

to the behavior problems of children
for professional and lay readers

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involved extremities was slightly increased at the most proximal joints, the more distal parts were flaccid. The arm was suspended at the animal's side and the leg was dragged passively (Expt 4)

2 When areas 4 and 6 (including 4s) were removed the contralateral hemiparesis was accompanied by flexion, pronation and adduction of the

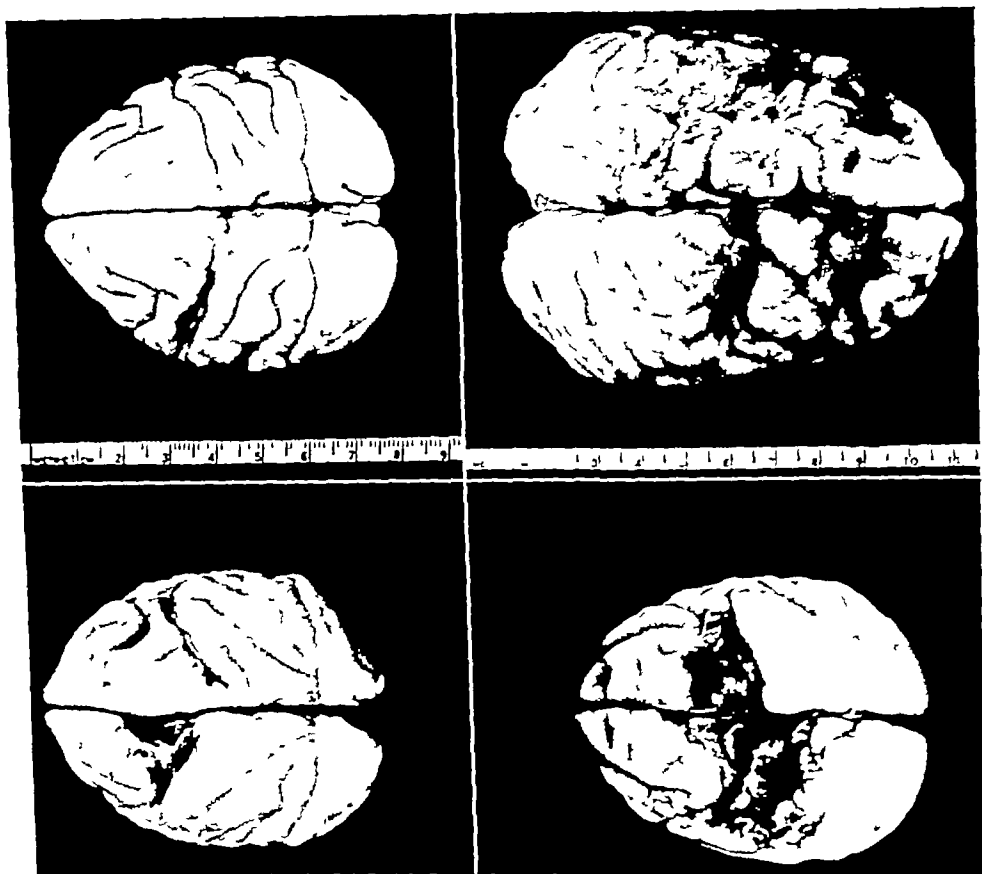


FIG 1 Brains of three monkeys and one chimpanzee showing discrete lesions Lt upper, Expt 1, rt upper, Expt 6, lt lower, Expt 4, rt lower, Expt 8

involved arm. Resistance to passive movement on the affected side was slightly or moderately increased and the tendon reflexes were frequently hyperactive. Forced grasping could, of course, be elicited. Ultimate recovery here was incomplete. The reflex grasp disappeared, but moderate paresis and slight increase in resistance to passive manipulation persisted (Expts 2, 4, 6)

3 Preexisting spastic paralysis was greatly accentuated by ablation of areas 4 and 6, either singly or together ipsilateral to the *initial spasticity* and

pyramidal areas posterior to the central sulcus from the standpoint of postural reflexes. Leyton and Sherrington (8) found no marked postural or motor changes following small lesions of the postcentral gyrus in chimpanzees. Hypotonia has been described as resulting from primary parietal ablation (7), but digital rigidity following a period of flaccidity was seen in two chimpanzees after large parietal ablations (12). When combined with destruction of the motor area, postcentral lesions have been known to cause spasticity in the chimpanzee (15, see reference 3, p. 410). But recently Browder reported a *reduction* in spasticity and resultant improvement in motor status in two of three hemiplegic patients when surgical ablation of the postcentral area was performed (1).

In the present experiments the effect on spasticity, flaccidity and paresis of single or combined ablations from the various parts of the sensorimotor cortex have been studied. Areas 6, 4 and the postcentral gyrus containing areas 3-1-2 have been removed in various combinations. No attempt was made to differentiate area 4s from area 6, and the two regions included together have been here designated as area 6.

MATERIAL AND METHODS

Fourteen primates were utilized, i.e., 9 *Macaca mulatta*, 3 *Cercopithecus torquatus* and 2 chimpanzees. Four other mangabeys were used for comparison, following bilateral removal of area 6 alone (16). The results were compared with the findings on a large number of animals previously operated upon in the Laboratory which had had primary ablations from these cortical areas.

All cortical excisions were performed with aseptic technique under sodium amytal anesthesia according to the method long in use in the Laboratory. Cortical tissue was dissected with a spatula or a pledget of moist cotton after thermocoagulation of surface vessels. Retraction was applied only to tissue which was to be excised and gentle suction was used to remove cortex adjacent to sulci so that the pia in the sulcus was not disturbed. Hemostasis was obtained by the use of electrical coagulation, silver clips and warm, moist patties. Only rarely was electrical stimulation used to delimit cortical areas. Instead, ablations were made according to landmarks on the surface of the cortex as shown in the illustrations (Fig. 1, 2). The division between areas 4 and 6 is always questionable unless identified by the technique of Dusser de Barenne and McCulloch using suppression as the quality identifying area 4s. Two types of ablation from area 4 were therefore made. One, a larger one extending through the excitable motor area of the precentral gyrus, the other, limited only to the anterior lip of the central sulcus.

Observations on gait, posture, motor and reflex status were made at frequent intervals before and after operation.

EXPERIMENTAL DATA

Because of limitations of space only abstracts of protocols of selected animals have been appended.

A Primary lesions

Primary lesions of single parts of the sensorimotor areas of the cortex were made in the initial stage of each experiment. The results of such lesions confirmed entirely the effects described by previous investigators following similar lesions (2, 4, 5, 6, 7). They are here summarized.

1. Excision of area 4 caused severe contralateral hemiparesis which improved gradually and incompletely. Resistance to passive movement of the

either of these lesions caused contralateral spastic paralysis as well (Expts 2, 4, 6,)

4 Following simultaneous bilateral destruction of area 6 or areas 6 and 4s four mangabey monkeys exhibited spastic paresis in flexion of all four extremities in addition to tremor. This has been described in detail elsewhere (16)

5 Primary injury to the parietal areas resulted in contralateral hemiparesis which was more severe in the chimpanzee than in the monkey. In the former animal there was complete flaccidity and recovery of movement occurred almost simultaneously in all parts. With motor recovery "tone" returned to normal and only slight residual paresis remained. In the monkey hemiparesis was accompanied by transiently diminished resistance to passive movement (Expt 3)

B Combined lesions

Successive or simultaneous ablations of the motor and sensory areas showed that, in monkeys, injury to the parietal lobe neither protected against subsequently induced spasticity nor caused flaccidity when the motor areas had been previously excised (Expts 1, 2, 3, 6). An exception to the latter point was, however, observed. One monkey showed flaccid paralysis when parietal ablation was superimposed on an old spasticity, more recently accentuated by ipsilateral area 6 ablation. It is probable that in this experiment, during the long period of recovery from the effects of the first operation, reorganization in the nervous system caused the response to parietal injury to simulate that of an intact animal. Most often preexisting spasticity was unaltered by subsequent destruction of the parietal areas in monkeys although a few animals showed slight change (Expts 1, 2, 3, 5). In the chimpanzee, however, frontal lobe spasticity and paresis were greatly intensified by subsequent postcentral excision and marked bilaterality of this effect was noted (Expt 6).

A more delicate test of the relation of the sensory areas to spasticity is the successive ablation of area 4 and the postcentral gyrus. When, in the monkey, postcentral ablation was performed subsequent to destruction of area 4 the contralateral arm became flexed, adducted and semipronated and resistance to passive movement at the intermediate and distal joints became increased after an interval of several weeks. The opposite experiment was performed in the chimpanzee. Here area 4 ablation was superimposed on ablation of the postcentral gyrus with precisely the same effect on posture and "tone" in the involved arm after a somewhat longer delay (Expts 1, 3).

Confirmation of some of these physiological findings appeared on histological examination of the degeneration in the direct corticospinal fibers. The degeneration of fibers in the pyramids and in the dorsolateral column of the spinal cord was very different in those animals having complete hemispherectomies from that in animals having removal of the motor areas only.

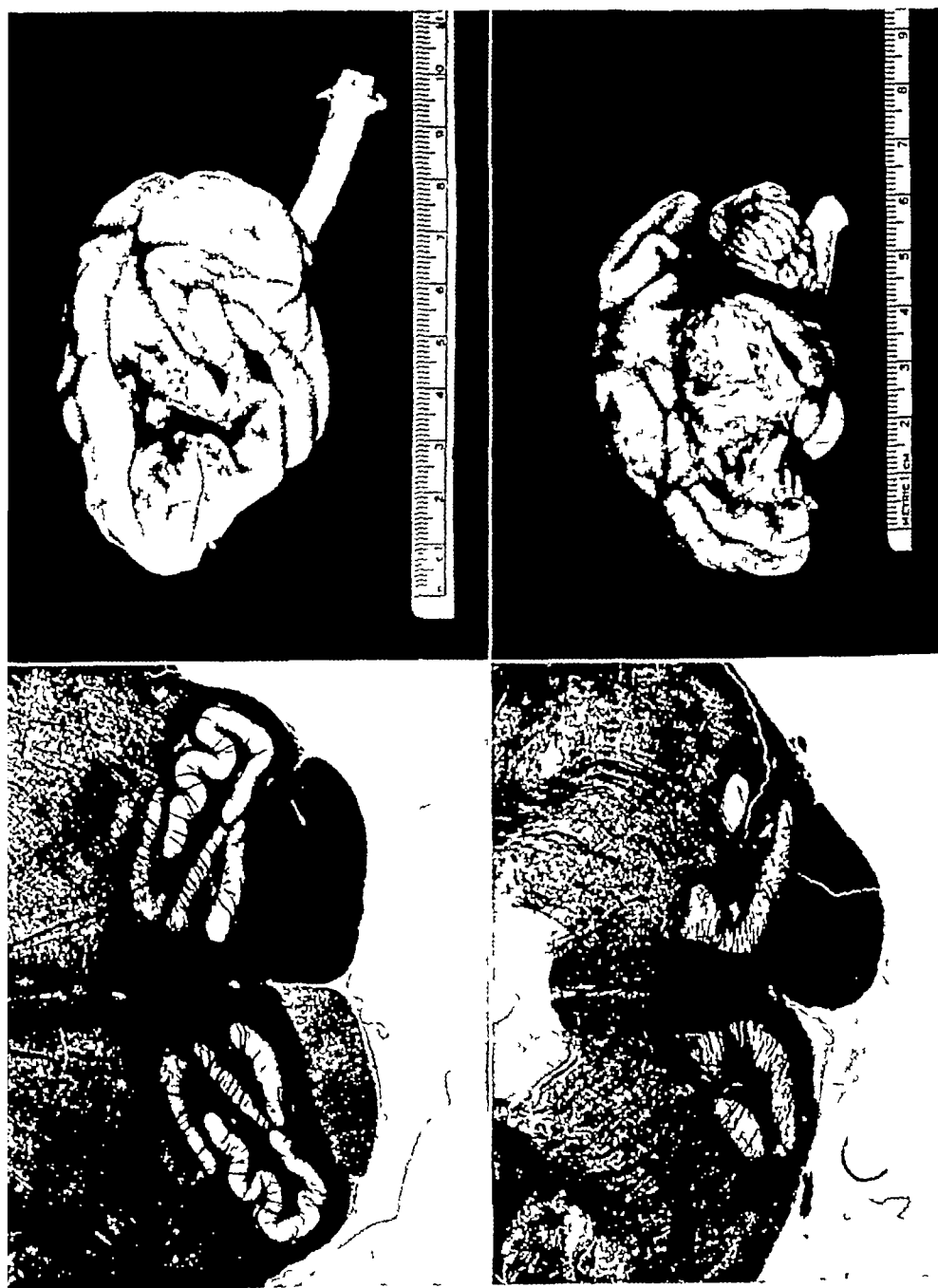


FIG 2 Comparative degeneration in the pyramids of two monkeys, one (upper) following ablation of areas 4 and 6 the other after total hemispherectomy Weigert stain

1 Damage to predominantly *extrapyramidal* cells is followed by moderate paresis with increased resistance to passive manipulation and hyperactive tendon reflexes

2 If areas predominantly *pyramidal* are then added, spasticity is increased because additional extrapyramidal fibers are injured and because the normal pyramidal influence on the final common pathway is removed as well

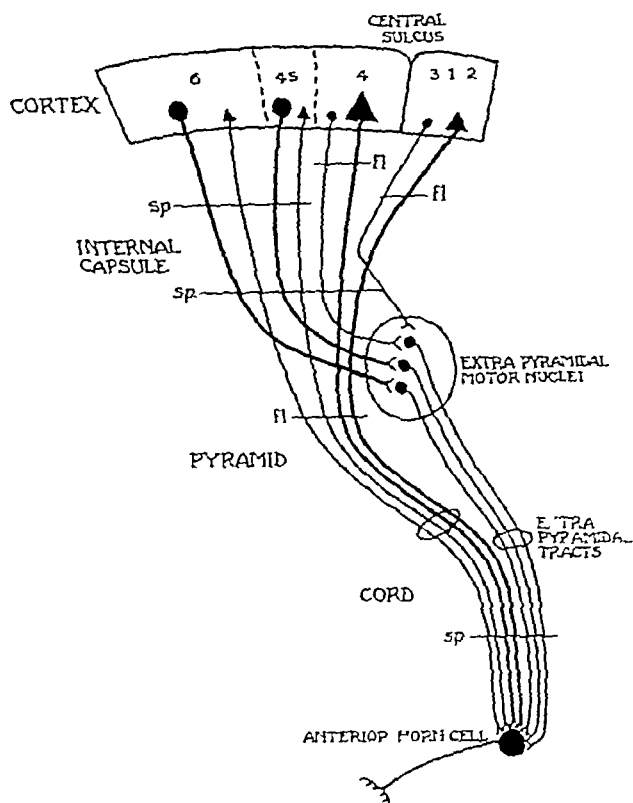


FIG 3 Diagram of pyramidal and extrapyramidal pathways The direct cells of the pyramidal tract are represented in the cortex as pyramids, those of the extrapyramidal as circles The size of these symbols represents relative number of cells in each cortical area

3 Since, in man, the pyramidal tract is relatively more important than in the monkey and since *cerebral dominance* is greater in the extrapyramidal sphere also, the flaccid stage which follows immediately after interference with direct corticospinal connections to anterior horn cells is more profound and enduring But, when this stage is past and the final common pathway becomes again capable of being influenced by the released, subcortical regions, spasticity from cortical injury becomes more pronounced in man than in the monkey

The findings of Browder, that postcentral ablation, in man, diminishes

Thus, in the medullary pyramid there was complete degeneration of all fibers when one hemisphere had been removed, but following extirpation of areas 4 and 6, the degeneration was only moderate (Fig 2) When the post-central gyrus was added to the motor areas, the degree of degeneration lay between the two above extremes In the dorsolateral tract of the cord removal of areas 4 and 6 caused relatively slight loss of active fibers and even hemispherectomy did not produce complete degeneration The existence within the "pyramidal tract" of the cord of extrapyramidal fibers with cells of origin below the cortical level is thus indicated by the difference between total number of fibers showing degeneration in the tracts at medullary and spinal levels in the same animal

DISCUSSION

The alterations in resistance to passive movement produced by specific cortical lesions in these monkeys and chimpanzees have been consistent throughout the series and agree with the observations of many previous investigators They therefore warrant consideration from the standpoint of their physiological significance In this investigation as in others, however, the difficulties of making categorical statements in the presence of findings which are both changing and variable must be recognized In the monkey, the change in posture, muscle and reflex status which follows circumscribed cortical injury are never extreme except during the first few postoperative days Increased resistance to passive manipulation of the extremities, for example, usually is slight or moderate in degree Furthermore, there is a certain range of variability in these changes both between different animals and in the same animal at different times It is a well recognized fact that the same variability is present in both anthropoid apes and man although symptoms are always more marked here, and the changes are magnified because the cerebral cortex is more highly and complexly organized

In Fig 3 are outlined the anatomical pathways necessary in order that cortical lesions may produce the states of flaccidity and spasticity which have occurred in the present experiments As shown in the diagram, the pyramidal fibers act directly upon the motor cells of the cord at or near the final common pathway, to direct the fine volitional muscle movements The extrapyramidal fibers, in contrast, act on sub-cortical centers of more complex function, integrating there the postural and locomotor adjustments When the predominantly pyramidal regions are excised profound alterations in the status of the anterior horn cells occur and flaccidity results unless the extrapyramidal regions have been injured also If this last occurs *after* the ventral horn cells have undergone "recovery" from the removal of pyramidal influence, both paresis and spasticity are augmented These differences and changes were found to be more definite in the chimpanzee than in the monkey and clinical material indicates that in man the effect of cortical lesions in producing spastic and flaccid paralysis is even greater It can therefore be hypothesized that in the monkey, chimpanzee and man, injury to the cortical motor areas is effective in the following manner

Postoperative notes 1st day The animal sat and stood with the right arm dangling at its side. The right leg was dragged in walking.

3d day There was marked impairment of movement on the right. The right arm was not used in walking or climbing but hung at the animal's side. The dorsum of the right foot was dragged along the floor in walking. No fine movements were seen on the right. There was slight increase in resistance to passive movement in the right extremities at the most proximal joints but in the distal joints resistance was diminished, the right knee jerk was more active than the left.

7th day There had been no change in postural, motor or muscle status.

14th day Movement had improved so that the cage side was sometimes grasped by the right hand in climbing. Posture was unchanged and the fingers of the right hand were relaxed, neither flexed nor extended. Muscle status was unchanged. The right knee jerk was more active than the left.

2d operation July 2, 1943 The left postcentral gyrus (areas 3, 1 and 2) was removed by subpial suction.

4th day Movement on the right was more severely impaired than before the second operation. Fine movements were not seen and the right arm and leg were frequently misplaced in walking. Resistance to passive movement was, as before, slightly increased in the proximal portions of the right arm and leg but decreased at elbow, knee, wrist and ankle. The right knee jerk was more active than before operation.

6th day For the first time the fingers and wrist on the right side showed an increased resistance to passive movement. The fingers were flexed at interdigital joints and neither flexed nor extended at the metacarpophalangeal joints.

Subsequent course The animal's right arm assumed the semiflexed "hemiplegic" posture but as movement improved this largely disappeared. Slight atrophy was noted in the right leg. The hyperactive right knee jerk persisted. Resistance to passive movement of the extremities returned to normal.

September 23, 1943 The animal was sacrificed and autopsy was performed. All viscera were grossly normal.

Anatomical note The brain weighed 100 gm. There had been clean ablations of the anterior and posterior lips of the central sulcus. Nissl sections showed a small circumscribed lesion involving only the gray matter about the central sulcus. Some Betz cells were seen both anterior and posterior to the lesion. Marchi sections revealed degeneration of one pyramidal tract in medulla, pons and cord.

Summary After ablation of area 4 on the left, this monkey showed a right hemiparesis with flaccid distal joints but slight increased resistance to passive movement in the most proximal ones. The right knee jerk was hyperactive. After ablation of the left postcentral gyrus there was delayed and transient increase in resistance to movement of the right distal joints and the arm, previously hanging straight at the side, assumed the semiflexed, "hemiplegic" attitude.

Experiment 2 First operation Ablation of lt areas 4 and 6. Spastic hemiparesis with recovery except for fine movements on rt side. *Second operation* Ablation of rt area 6. Accentuation of rt paresis and addition of slight paresis on lt. Transient increase in resistance to passive movement in all extremities. *Third operation* Ablation of rt postcentral gyrus. Accentuation of lt paresis with addition of proprioceptive deficit. Slight transient increase in spasticity (R S 23).

1st operation May 3, 1943 Areas 4 and 6 on the left were excised.

Postoperative notes 7th day The right hand and foot were used infrequently and poorly and the toes on that side often became caught in the wire cage bottom. Rossolimo's sign was elicited on the right. The left knee jerk was more active than the right.

15th day The animal was active but did not use the right arm for support. The right extremities were held slightly extended and the right knee jerk was more active than the left.

22nd day The affected extremities were used fairly accurately and with speed. They could be used for the animal's sole support. Fine movements were, however, deficient. Rossolimo's sign was demonstrated on the right and slightly increased resistance to passive movement of the right extremities.

preexisting spasticity must be explained by this fact of relative dominance of the pyramidal system. It would account for a temporary flaccidity but not for an enduring one unless the final common pathway is so affected by pyramidal injury that complete paralysis and immobility of the limb results. In the chimpanzee such a procedure caused, eventually, greater spasticity than in the monkey. The histological data have been offered here merely as confirmation of the fact that neither pyramidal nor extrapyramidal fibers are confined to the true motor areas. All evidence presented is thus in harmony with the statement of Fulton (3) who writes "the intensity and duration of spastic resistance are functions, not of any area, but of the extent of interruption of the extrapyramidal cortical projections."

SUMMARY

Observations on the state of spasticity or flaccidity have been made in monkeys and chimpanzees following cortical ablations

- 1 Primary removal of the various cortical areas has confirmed observations made by previous investigators
 - a Removal of area 6 (including area 4s) was followed by moderate paresis which is spastic in nature
 - b Removal of area 4 or of the anterior lip of the central sulcus was followed by paresis without spasticity
 - c Removal of the postcentral gyrus or of the posterior lip of the central sulcus caused flaccidity, transient and with some paresis. Symptoms were more marked in the chimpanzee than in the monkey.
- 2 Successive removal of various of these combined areas caused changes as follows
 - a The ablation of areas 4, 4s and 6 either together or seriatim in any sequence resulted in immediate spastic paresis
 - b The addition of the postcentral gyrus to ablation of any areas producing spasticity increased paresis. Spasticity was usually increased in the monkeys, although this was not as marked. In the chimpanzees, after a short initial period of marked flaccid paresis, definite spasticity appeared
 - c Combined lesions of area 4 and the postcentral gyrus result in spasticity
 - d The addition of any contralateral motor area to a primary lesion causing spasticity increased this spasticity
 - e Removal of an entire hemisphere secondary to ablation of areas 4 and 6 in the monkey augmented the spasticity and paresis to greater degree than did ablation of the postcentral gyrus

ABSTRACTS OF PROTOCOLS

Experiment 1 1st operation. Ablation of lt area 4. Right hemiparesis with improvement 2d operation. Ablation of lt postcentral lip. Slight accentuation of paresis and increase in resistance to passive movement of rt extremities (R S 34)

1st operation. June 18, 1943. A subpial ablation of area 4 on the left was performed

4th day The right arm and leg were still not moved spontaneously and that side of the body was flaccid

7th day For the first time since operation the animal stood up. This was accomplished by supporting itself with the left hand. The right arm was held partially flexed. Climbing was also done but all the strength and movement was supplied by the left arm and leg. Some slight resistance was met in moving the right extremities.

8th day The right hemiparesis continued but now the animal occasionally moved the arm. Movements of the fingers were performed as well as gross movements. Weight was not borne well on the right leg and this member often assumed a grotesque position. The right arm and leg were flaccid except during apparently voluntary movement. A positive Babinski reflex was demonstrated.

10th day Motor ability was improved in the right arm so that it was able to grasp food with the hand and carry it to its mouth. Resistance to passive movement was less on the right than on the left side.

14th day No change since the last examination.

Interval course Improvement continued. Posture and muscle status became normal on the affected side. The only residues were dorsiflexion of the right toes, slight dragging of the right leg in walking and the use of the left hand in preference to the right. Resistance to passive manipulation continued less on the right than on the left side in both arm and leg. The proprioceptive deficit which had been previously demonstrated by the animal in feeding and maintaining posture became undetectable except that occasionally the right arm and leg would be held in unusual positions.

2d operation July 20, 1943. The entire area 4 on the left was excised.

Postoperative notes *1st day* The animal lay on the right side but sat up when approached. The right extremities were nearly flaccid and no movements were seen on that side.

2d day Large movements of the proximal joints were now performed on the right and these extremities were flaccid. The arm hung limply at the animal's side.

7th day There was further improvement in motor status, i.e., walking and climbing were now performed. The right extremities did not bear weight in climbing, however. Resistance to passive movement was about normal on the right.

Interval course Improvement continued and the right hand was used in eating when the other hand was occupied and for climbing and scratching. The right knee jerk was more active than the left. On the *27th day* it was noted that the right arm was frequently held flexed, adducted and semipronated and that resistance, particularly to extension was increased. When last examined *April 22, 1944* the only postural deficit was slight hyperextension of the right metacarpophalangeal joints. When excited, however, the right arm reverted to the flexed, adducted and semipronated posture. Resistance to extension of the right arm was slightly increased.

Summary Right flaccid hemiparesis resulted from ablation of the left postcentral gyrus in this chimpanzee. Recovery occurred almost simultaneously in all parts of the body and was nearly complete. Resistance to passive movement of the extremities gradually returned to normal. Subsequent ablation of area 4 on the left caused hemiparesis, initially flaccid but after nearly 4 weeks spastic in nature. Resistance to passive manipulation appeared in the right arm and that member assumed the "hemiplegic" attitude.

Experiment 4 *First operation* Ablation of lt areas 4 and 6. *Rt spastic hemiparesis with gradual and incomplete recovery.* *Second operation* Ablation of rt area 4. *Bilateral spastic hemiparesis with greatest spasticity on rt (R S 21)*

1st operation April 16, 1943. Areas 4 and 6 were excised on the left.

Postoperative note *2d day* No voluntary movements were observed in the affected extremities. The right arm was semiflexed and the right leg, flexed. Resistance to passive movement of the right extremities was slightly increased.

3d day The right arm was held in acute flexion and showed increased resistance to movement. The right leg was not spastic, however.

Interval course Movement gradually improved but no fine movements were noted. Forced grasp and Rossolimo's sign were present on the right. Spasticity never became marked in the leg but continued for a time in the right arm. Knee jerks were active.

Interval course Improvement continued but no fine movements were seen on the right and the left extremities were used in preference where this was possible. Reflex grasp was present in hand and foot. The knee jerks were equal and resistance to passive movement returned to normal.

2d operation June 22, 1943 Area 6 was removed on the right.

Postoperative notes *2d day* The animal was generally hypoactive. The left hand was used in preference to the right but finger movements in both hands were not accurate. Climbing was impeded by reflex grasp on the left side. Resistance to passive movement of the extremities was normal or slightly increased bilaterally, the right knee jerk was more active than the left, reflex grasp was present bilaterally.

3d day Postural, motor and reflex status was unchanged except that there was a definite increase in resistance to passive movement of all extremities.

4th day Activity had increased but no definite changes were noted.

Interval course During the next two weeks the bilateral paresis continued. Increased resistance to movement of the extremities continued, was variable, then disappeared on the right but remained to slight extent on the left. The right knee jerk remained more active than the left.

3d operation July 9, 1943 The right postcentral gyrus was ablated.

Postoperative notes *3d day* The left arm was held flexed, pronated and adducted. Movement was greatly impaired and proprioceptive deficit was marked. There was left homonymous hemianopia and diminished response to tactile stimulation on the left. There was no increase in resistance to passive movement of the extremities. Knee jerks were active and equal.

4th day In walking the extremities were raised high off the floor. Resistance to movement of the left extremities was now increased and the left knee jerk was more active than the right.

5th day The increase noted in resistance to passive movement of the left extremities was now only slight and the knee jerks were equal.

Interval course Movements showed some improvement but the gait was characterized by hopping and overstepping, climbing was impeded by prolonged grasp. The knee jerks were equal and active and resistance to manipulation was not increased in the extremities. The legs were held flexed when the animal was in the sitting posture.

September 25, 1943 The monkey was sacrificed.

Anatomical note The brain weighed 85.4 gm. The operative lesions were discrete except that area 4 had been slightly damaged on the right.

Summary This animal showed right hemiparesis, reflex grasp and spasticity following ablation of left areas 4 and 6. Improvement was gradual and incomplete. Ablation of right area 6 was followed by left hemiparesis with transiently increased resistance to passive movement on that side and temporary accentuation of the right spastic hemiparesis. Subsequent ablation of the right postcentral gyrus resulted in increased paresis and great proprioceptive deficit on the left and in slight and transient increase in spasticity on the right.

Experiment 3 *First operation* Ablation of lt postcentral gyrus. *Left flaccid hemiparesis with almost complete recovery*. *Second operation* Ablation of rt area 4. *Initial flaccid hemiparesis followed by mild spasticity in rt arm* (C 108).

1st operation May 5, 1943 A large piece of postcentral cortex weighing 6.2 gm was removed. The dissection was carried to the depth of the interparietal sulcus and was extended over the medial surface of the hemisphere to include all the postcentral gyrus. At operation corticopial adhesions about the size of a nickel were seen over the frontal cortex, possibly indicating previous trauma, although no bone fractures could be seen.

Postoperative notes *1st day* The animal lay on its back and the right extremities would fall wherever they were placed. Few movements were performed. The left hand accepted food and carried it to its mouth. The right arm and leg were flaccid and the knee jerk was not elicited on that side.

3d day Right flaccid hemiplegia continued. Movements on the left were well performed but were slow.

Summary Following ablation of areas 6, 2 and 5 on the left this monkey showed mild spastic paresis in the contralateral limbs. Further injury to the left parietal lobe caused transient accentuation of the proprioceptive deficit but did not modify muscle status.

Expt 6 First operation Ablation of lt area 4 and 6 *Right spastic hemiplegia with partial recovery* *Second operation* Ablation of rt area 6 and part of 4s *Left hemiparesis and accentuation of rt paresis and spasticity* *Improvement* *Third operation* Removal of lt parietal areas. *Increase of paresis and spasticity on rt* *Fourth operation* Removal of rt parietal areas. *Quadriplegia with spasticity most marked on rt* (C E)

1st operation November 14, 1938 After stimulation of the cortex, tissue corresponding to areas 4, 4s and 6 was excised on the left.

Postoperative notes *Day of operation* There was deviation of the head and eyes to the left. The right arm was held adducted and flexed and the wrist was flexed. The right leg was flexed also.

2d day Resistance to manipulation of the extremities was increased on the right side. Paresis on the right was profound. The posture of the arms and legs remained the same. Reflex grasping was demonstrated in the right hand and foot. The head and eyes were no longer deviated.

4th day Movement was improving but the animal was not able to bear weight on the right leg. Posture and muscle status were unchanged.

5th day Posture was unchanged and movement was slightly improved. The arm was still not used well. Resistance was a little greater on the right than during the last examination. The knee jerks were equal and toe jerk was more pronounced on the right. There was reflex grasping on the right.

Interval course There was a gradual improvement in the hemiparesis. Tendon reflexes became increased on the right and remained so. Posture on the right became nearly normal but the toes on that side remained plantar flexed and when the animal became excited the right arm assumed the flexed, adducted and semipronated posture. Increased resistance to passive movement on the right gradually decreased but did not entirely disappear. Fine movements never returned to the right hand and the left was used instead for eating and scratching. Atrophy in the right arm and leg was marked.

2d operation April 12, 1943 Area 6 and part of 4s were removed on the left.

Postoperative notes *1st day* There was left hemiparesis and spasticity on the right was greatly accentuated. Reflex grasp was demonstrated in the right hand and foot. The knee jerk was active on the right, absent on the left.

2d day The animal was hypomotile and sat in the cage with legs partially flexed. It was disinclined to bear weight on the right leg. Resistance to passive movement of the right arm and leg was extreme, on the left side it was moderately increased.

3d day There was little change. The right arm was now held flexed. The right knee jerk was more active than the left and Hoffman's and Rossolimo's signs were elicited on the right side.

Interval course Movement improved and in the left hand fine movement reappeared. Resistance to passive movement of all extremities became less marked so that on the right side it was slightly to moderately increased and on the left it was normal.

3d operation May 17, 1943 The left areas 3, 1, 2, 5 and 7 were removed. Area 8 was slightly injured because of dural adhesions during the elevation of the bone flap.

Postoperative notes *1st day* There was deviation of the head and eyes to the left and slight right facial weakness. The right limbs were held in grotesque positions and on that side movements were poorly performed and weight was not borne. The right arm and leg were but slightly resistant to passive movement.

2d day The right arm and leg were now greatly resistant to passive movement and the right arm was held in the "hemiplegic" attitude.

Interval course Movement improved and the spasticity on the right became less marked but remained.

4th operation July 29, 1943 The right postcentral gyrus was removed.

Postoperative notes *3d day* The animal lay in the cage and was only able to roll over to right or left and partially sit up by supporting itself on the cage side. The arms were held flexed and the fingers on the right were extended. Resistance to passive movement was extreme on the right and moderately severe on the left.

2d operation June 9, 1943 Area 4 on the right was excised

Postoperative notes 1st day The animal sat in a half kneeling position with legs flexed. Occasionally the dorsum of the left foot was used to bear weight. Several small, slow steps were taken. Resistance to passive movement was increased in all extremities and this was most marked on the left. Forced grasp was present bilaterally.

2d day Walking could not be performed without falling. Resistance to movement of the legs was increased but not as much as on the preceding day. Placing responses could not be elicited.

5th day The ability to stand and walk had been lost. The animal lay on either side with the undermost leg extended and the uppermost extremities flexed. On the left spastic resistance was not increased, on the right, slightly so. Knee jerks were equal.

6th day The right extremities were now markedly resistant to passive movement and on the left resistance was slightly increased.

June 22, 1943 Autopsy Both lungs showed extensive areas of nodular hardening and the hilar lymph nodes were enlarged apparently from tuberculosis.

Anatomical note The brain weighed 78 gm. The operative lesions were confirmed grossly.

Summary This animal developed right spastic hemiparesis following ablation of left areas 4 and 6. The arm was more severely spastic than the leg. Recovery was gradual and fine movements did not return. Subsequent ablation of area 4 on the right caused accentuation of paresis and spasticity on the right and added left spastic hemiparesis.

Experiment 5 First operation Ablation of lt areas 6, 2 and 5. *Paresis, proprioceptive deficit and increased resistance to passive movement on rt.* *Second operation* Ablation of lt posterior parietal cortex. *Transient increase in proprioceptive deficit. Increased resistance to passive manipulation not changed by operation (R S 30)*

1st operation June 4, 1943 The left hemisphere was exposed and areas 6, 2 and 5 were removed. In order not to damage area 4, the posterior lip of the central sulcus—largely area 3,—was left intact.

Postoperative notes 1st day The right arm was held stiffly extended and was not used in voluntary movement. Weight was borne on both legs. Response to touch was diminished on the right side.

3d day The animal sat in the cage with the right arm partially flexed. The forearm, hand and fingers on the right were held in the same plane. Only gross movements were performed on the right. Resistance to passive movement of the right arm and leg was increased and the right knee jerk was more active than the left.

6th day Resistance to passive movement of the right arm was still increased but both legs showed normal resistance.

8th day Posture and movement had not changed. Resistance to passive movement was now increased in all extremities but this was more marked on the right.

11th day Skill had improved. Resistance to passive movement was increased in all extremities but this was more marked on the left.

2d operation June 17, 1943 The posterior portion of the left parietal lobe, areas 5 and 7, was removed.

Postoperative notes 1st day A great increase in proprioceptive deficit was observed on the right. There was a right homonymous hemianopia. Resistance to passive manipulation was slight on the right.

4th day The animal sat with the right arm semiflexed. All extremities were resistant to movement but this was slight on the right.

8th day No change in muscle status had occurred. The right knee jerk was exaggerated.

16th day Proprioceptive deficit was less marked and the animal ran and climbed with agility. The right arm showed increased resistance to passive movement. The legs showed equal resistance.

September 23, 1943 The animal was sacrificed and autopsied. All viscera were normal.

Anatomical note Examination of the brain confirmed the operative lesions.

ACUTE AND CHRONIC PARIETAL LOBE ABLATIONS IN MONKEYS

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INTRODUCTION

ANALYSES of parietal lobe functions in animals have for the most part been made without regard to limits of individual parietal areas, and have been based chiefly on results of ablations of gyrus or of greater amounts of cortical tissue. In the macaque five areas in the parietal lobe having discrete cyto-architectural characteristics, as well as differences in their afferent and efferent fiber connections, have been described (19, 26). Hughlings Jackson believed that such difference in structure indicated differences in function. Studies of physiological disturbance resulting from removal of the individual parietal areas should then prove of interest. Such studies in macaques form the basis of this report and include observations in "acute" and "chronic" preparations.

Among the earliest reports of experimental ablations of the parietal lobe are those of Grunbaum and Sherrington (9, 10). They found that large ablations of the postcentral gyrus of the higher apes did not result in paralysis. Brodmann (2) also failed to note paralysis, but mentioned hypotonia after postcentral lesions in *Cercocebus* monkeys. Vogt (25), Lewandowsky and Simons (15), and Rothmann (20) studying effect of postcentral lesions in macaques, described ataxia and disturbed sensibility without paralysis, but with a reluctance to move the limbs opposite the lesion. Rothmann in addition described awkwardness of the contralateral hand and fingers and an initial diminution of tactile sensibility following extirpation of the "supramarginal" gyrus (area 7). Minkowski (17) using *Macaca mulatta* performed several types of parietal ablations and concluded that tactile sense and deep sensibility were represented pre- and postcentrally, that cutaneous sensibility providing discrimination was represented in the postcentral gyrus chiefly but also in other parietal areas, and that pain sense and its localization had an extensive cortical representation. Ruch and Fulton (21, 22), in the course of an investigation in monkeys, and chimpanzees and man of cortical representation of the ability to discriminate small differences in the magnitude of lifted weights, suggested that the inferior parietal lobule represents the highest level of sensory integration. Ferraro and Barrera (6) removed "postcentral convolutions" from ten monkeys (*M. mulatta*) and described early impairment of sense of position and of movement, hypotonia, apparent increase of deep reflexes, and loss or diminution of the hopping and placing reflexes. Kennard and Kessler (14) in their report of both unilateral and bilateral parietal ablations, described a "motor deficit," characterized by disuse,

4th day Movements were gross and inaccurate bilaterally Muscle status had not changed

5th day Standing and walking could now be performed with support There had been no other change

Subsequent course Motor status improved so that walking was performed with less and finally without support Individual finger movements reappeared on the left but only gross movements were ever seen on the right Resistance to passive movement remained increased on the right but on the left became less marked Deep reflexes were hyperactive bilaterally

September 28, 1943 The animal was sacrificed and autopsy was performed All viscera were normal

Anatomical note The operative lesions were confirmed by gross and histological examination In addition there was a small area of softening in area 8 on the left

Summary This immature female chimpanzee showed right spastic hemiplegia following ablation of areas 4 and 6 on the left Recovery was gradual and incomplete Five years later area 6 and part of 4 F were excised from the right cortex causing left spastic hemiparesis and transient accentuation of paresis and spasticity on the right Left parietal ablation caused a further increase in paresis and spasticity on the right side Following ablation of the right postcentral gyrus all extremities showed extreme paresis and spasticity which were more severe and persistent on the right

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passively irresponsive to even severe stimuli, while again they responded quickly to mild stimulation. Repetition of the examination under good emotional conditions with repeated similar results served to indicate the changes present after parietal ablation. Examination of the sensory status included response to pin prick, to tactile stimuli using cotton or food particles, and the response to abnormal passive posturing of the limbs (lower). The response to pin prick was evaluated either by facial grimace, withdrawal of the part stimulated or use of warding off movements with the limbs, or initiation of scratching in the vicinity of the stimulus. Appreciation of localization of stimuli was evaluated by the locus of an area scratched in response to a pin prick, and by the grasping of food particles and other objects brought into light contact with parts of the monkeys' limbs.

The hopping and placing responses, proprioceptive and tactile, as outlined by Bard (1) were tested in all animals, visual aid was avoided by use of a blindfold. It was hoped that these reactions would give information concerning possible differential localization of tactile and proprioceptive stimuli. Proprioceptive placing reactions studied were those in response to displacement of the limb, upper or lower, by bringing the limb in contact with a sharp table edge. Tactile placing reactions involved contact between the dorsum of the hand or foot and a tuft of cotton, placed on a table edge. Examinations were carried out in a quiet room and all phases of them were repeated several times.

RESULTS

Electrical stimulations

The electrical stimulations were useful in defining the areas in a general way. Thus, it was usually possible to delimit areas 5 and 7 from areas 3-1-2, by the type of movement elicited and the amount of current necessary to induce it. Areas 3-1-2, however, were not distinguished from one another, for, the movements obtained from each of these three areas and the strength of the current necessary to elicit such movements were similar. Flexion movements of the contralateral digits and/or of the distal joints of the limbs were usually obtained from areas 3-1-2 by stimulations with 4 volts E M F. Points over the postcentral gyrus, and especially area 3, from which movements of certain groups of muscles were elicited, bore a fairly close relation to points over area 4 from which contraction of similar muscle groups were obtained. A greater strength of current was necessary to elicit movement from areas 5 or 7 than from those three areas of the postcentral gyrus. The movements elicited from areas 5 and 7 were either elevation of the contralateral shoulder (sometimes both shoulders) and/or protraction of the entire upper limb. Movements of the thigh were not studied. Contraction of muscles of the shoulder girdle were obtained only from the anterior part of area 7 and the inferior part of 5 which lies along the anterior lip of the intraparietal sulcus. In those animals from which area 3 had been removed, subsequent stimulation of area 4 elicited normal contralateral movements.

Cytoarchitecture and degenerations

In the earlier report the cytoarchitecture of the parietal areas was described and a map presented. This was similar in most respects to Brodmann's (3) map for *Cercopithecus*. Cytoarchitectural description will not be repeated here, but when necessary allusion will be made to the previous report. The rapid myelin technique showed no efferent projections not already seen with the Marchi stain but rather verified in every case the previous observations. Gliosis was helpful in following the degeneration of fibers. All the parietal areas have been shown to send association fibers to the adjacent cerebral lobes, all sent many commissural fibers by way of the corpus callosum to the symmetrical parietal areas and

diminished resistance to passive movement, nonpersistent hyporeflexia, and awkwardness of movement, with a diminution of tactile and proprioceptive senses, as demonstrated by placing and hopping responses, particularly The larger the ablation the more serious was the "motor deficit" Paralysis has in no instance thus been found to follow parietal extirpations of varied amounts, certain losses in response to tactile and proprioceptive stimuli result, and such loss presumably accounts for awkwardness of movement

MATERIAL AND METHODS

The present report embodies observations of eighteen young monkeys (*Macaca mulatta*), in which discrete removals of parietal tissue were performed (see Table 1) Ether anaesthesia was used for the operations and the cortical tissue was carefully removed by the use of a cataract knife and small spatula The limits of the areas to be removed were determined in the early operations by use of Brodmann's (3) maps for *Cercopithecus*, subsequent studies of animals early in our own series showed no reason for changing the limits of the area to be ablated The area or areas to be removed were usually stimulated briefly with 60 c per sec sine wave current (18) as an additional aid in determining the limits of the ablation Area 4 was stimulated after making the parietal lesion to rule out any damage to motor cortex The largest ablations included areas 2, 1, 5 and 7 (lateral hemispheric surface), areas 1 and 2 were removed together, area 3, area 5 and area 7 (lateral hemispheric surface) were removed individually

Table 1 Table showing type of ablation and duration of observations

Animal	Date of operation	Area (s) removed	Duration of observation	Animal	Date of operation	Area (s) removed	Duration of observation
P1	20 May 40	1, 2, 5, 7	21 days	P12	1 May 41	7	375 days
P3	27 May 40	5	21 days	P13	10 Sept 41	5	369 days
P4	3 June 40	3	21 days	P14	18 July 41	1-2	21 days
P5	5 June 40	7	21 days	P15	6 May 41	1, 2, 5, 7	21 days
P7	16 July 41	3	21 days	P17	26 June 41	1-2	368 days
P8	27 Mar 41	1-2	21 days	P18	29 May 41	5	21 days
P9	1 Apr 41	1-2	21 days	P19	19 June 41	1, 2, 5, 7	368 days
P10	9 Apr 41	5	123 days	P20	14 Nov 41	1, 2, 5, 7	21 days
P11	21 Apr 41	3	381 days	T3	6 Jan 43	7	270 days

Eleven macaques forming an "acute" series were sacrificed after three weeks, and anatomical studies of nine, with the use of the Marchi and Nissl techniques, were reported previously (19) One animal died accidentally after four months, one was sacrificed after nine months, and five were killed after one year The rapid myelin stain (23) was used in the study of these last five "chronic" monkeys, one representing each type of ablation This technique was supplemented at times with the cresyl violet stain for nerve cells

Examinations and observations were similar for all eighteen animals They were observed frequently while they were free in their cages Their ability in climbing steps and in walking and climbing an extended rope, when restrained by the upper or lower limbs respectively, was also tested A neurological examination was done at regular intervals Spontaneous movements were closely followed, movements of the digits individually and together and of small muscle groups were particularly examined, co-ordinative movements were observed and associative movements were sought The condition of skeletal muscle "tone" was carefully noted (resistance to palpation and to passive movements) Reflexes, tendon and superficial, were studied These were tested while the animal was seated in the chair, and observations were made on the biceps, triceps, perosteal-radial, knee, ankle, finger, toe, abdominal, and in some cases, the Gonda (8) toe reflexes As is characteristic of monkeys, the emotional status of the animal at the time of the examination greatly influenced the results obtained in a sensory examination Some animals at times would sit

formance with vision Sweeping movements were frequently successful in getting food particles into the palm, but then poorly gauged individual contractions of the digits came into play and the food slipped through the fingers The affected hip, knee, and ankle were frequently flexed more than necessary in stair climbing, dorsiflexion was insufficient, especially at the ankle, to prevent the foot striking the step Too much protraction at the hip or flexion at the knee was noticeable in walking and excessive internal rotation of the thigh and abduction of the hallux caused the latter to strike the normal leg Searching for the rope in rope-walking or rope-climbing was seen and the foot or hand either overshot or underreached the rope Visual attention corrected all these faults, to the extent that the desired object was reached satisfactorily, though not as quickly as by the normal limbs Rage or pleasure (as in grasping food particles), however, increased the accuracy and speed of the movement, perhaps through more careful visual attention Ataxia in face and head movements was not observed

C *Associated movements* Associated movements of the affected limbs were observed frequently while the monkey was sitting in the chair These always accompanied strenuous contractions of the normal limbs by which the animal was warding off the examiner or trying to gain something beyond reach The associated movements were not complete mirrors of the purposive movement, rather partial patterns of those movements An extension-abduction movement of the normal upper limb, for example, was accompanied by weak abduction of the affected shoulder, the arm usually being only partially extended at the elbow and the hand extended at the wrist and fingers Protraction and kicking movements of the normal lower limb were accompanied by weak protraction of the affected lower limb

D *Position of extremities at rest* When the animals sat quietly, the affected extremities assumed "the parietic posture" The upper limb drooped at the shoulder, the arm was adducted, rotated medially, the elbow slightly flexed, the wrist loosely extended and the digits weakly and loosely flexed at the metacarpophalangeal and interphalangeal joints The lower limb was extended loosely in front of the animal, partially flexed at the knee, weakly rotated (or falling) inward, and plantar-flexed at the ankle, the foot was slightly inverted and toes plantar-flexed

II *CONDITION OF "TONE" IN SKELETAL MUSCLES* A *Resistance to passive movement and to palpation* An hypotonia of the contralateral muscles was consistently present after removal of the parietal areas This was noticeable in the animal at rest, and was also observed by testing the resistance to palpation and to passive movement The distribution of the greatest hypotonia was discernible at rest in "the parietic posturing" assumed by the macaque especially during the first few post-operative days This posture has been described above The posture and the resistance to passive movement indicated that hypotonia though present in all muscles was not equally distributed Proximal muscles were more hypotonic than distal muscles The elevators and abductors of the shoulder, the external rotators of the upper arm, the extensors of the arm, the flexors of the wrist and fingers, and the extensors and adductors of the fingers were especially hypotonic in the upper limb The flexors and extensors of the thigh and leg were about equally hypotonic The external rotators of the thigh, the dorsi-flexors (extensors) of the foot, and the abductors and extensors of the toes were more hypotonic than their antagonists The muscles of the leg were usually more hypotonic than those of the arm The trunk muscles were not noticeably hypotonic The hypotonia observed immediately after ablation persisted unabated throughout the period of observation

B *Reflexes, tendon and superficial* Tendon reflexes were consistently changed after removal of the parietal areas They were characterized by an increase in threshold, and in latent period, and by an increase in scope or excursion, and a slowness of execution All tendon reflexes could be described as pendular In all the characteristics above the knee jerks were altered more than the others, or at least alteration was more easily demonstrated here All these changes of tendon reflexes persisted throughout the three weeks' observation period The Gonda reflex was not present in the animals in which it was tested

Abdominal reflexes remained normal after all lesions of the parietal lobe

III *SENSORY LOSS* A *Response to varied stimuli* Response to pin prick was always present even after large parietal ablations, but was delayed and required a more intense stimulus over the contralateral half of the body More striking than the delayed response was the difficulty in localizing the stimulus This was shown by the frequent scratching by the normal or affected hand of a skin area on the affected side either proximal or distal

fewer fibers to non-homologous areas. All sent fibers to the *n. lateralis posterior*, *n. ventralis posterolateralis* and *n. ventralis posteromedialis* of the ipsilateral thalamus, fibers from rostral parietal areas terminated rostrad in these nuclei. A few fibers originating in area 5 ended in the *n. medialis dorsalis*. All areas contributed fibers to the pontine nuclei of the same side, and also fibers via the pyramid to the spinal cord in which they accompanied the lateral corticospinal tracts of the same and the opposite side, crossed fibers being more numerous. Areas 3-1-2, and to a less extent area 5, projected to lumbar levels of the opposite side, area 7 sent fibers to cervical levels only, area 3 sent a few fibers to ipsilateral lumbar cord levels. Fibers originating in areas 3 and 5 terminated in the rostral third of the lateral zone of the substantia nigra. Areas 5 and 7 sent fibers to the pretectal region, and area 7 sent a few into the superior colliculus.

Functional deficits

Removal of all areas except 3. Four animals, P1, P15, P19, P20. Their early post-operative behavior was similar.

"ACUTE"

I. MOTOR PERFORMANCE. *A. Spontaneous use of skeletal muscles.* Voluntary movement in the limbs contralateral to the lesion was at a minimum during the first three to four days. These extremities were rarely used except in locomotion or as support. As early as three hours post-operatively, however, one animal was observed to wipe his face with the upper limb, the shoulder was abducted, the elbow and wrist joints were flexed and the fingers were flexed laxly into the palm, the entire limb being used somewhat as a unit. Food placed in the hand contralateral to the lesion was often removed by the normal one and except as a necessity the latter executed all feeding performance. The dearth of movement was not due to paralysis, for if the normal limbs were restrained and some interesting food article proffered, the monkey usually reached for it. In climbing and in ordinary progression all extremities were readily used. The monkeys appeared loath to move the limbs opposite the lesion, rather than to have a paresis.

Differences in the performance of the limbs contralateral to the lesion and that of the normal extremities were noticeable. Ataxia or an awkwardness of movement, a laxity and a slowness of movement were particularly noticeable. Individual use of the digits as well as movements of small muscle groups were possible. Grains of rice and bits of raisins could be picked up, but if some sweeping movement of the hand and fingers as a unit would suffice, or if the food could be reached with the head and teeth, one of these movements was used. If the digits were used individually, and if the object were on a flat surface, the grain was obtained by opposition of the pad of the thumb against the radial side of the index finger near the base of the terminal phalanx. Opposition and flexion of these two digits were used in obtaining grains placed between the fingers of the examiner. At the shoulder the necessary elevation, abduction or rotation was performed and at the elbow joint, flexion or extension accompanied the hand movement. The limbs were not used as single units, but muscle groups were used synergically. Movements of the shoulder muscles were slower and seemed to require more effort than those of the wrist and fingers. All movements were executed more quickly towards the end of the three weeks' observation period although at this time the activity of the affected limbs lagged behind that of the normal side.

In climbing, the affected toes and fingers grasped the wire, but the hold was looser than that of the normal digits. Stairs were climbed by the appropriate movements at the hip, knee, and ankle joints, rather than by movement of the entire limb as a unit. Flexion of the hip, knee, and ankle alternated properly with extension of these joints. The toes were loosely flexed or extended. In rope walking, displacement of the foot through its contact with the rope sufficed to bring about grasping of the rope by the affected toes but again the grasp was looser than that of the normal digits and frequently consisted of an adduction of any two digits between which the rope happened to strike. This latter position was not usually corrected quickly, occasionally, though, the hallux and other digits wrapped around the rope from opposite sides.

B. Ataxia. Ataxia in the movements of the limbs contralateral to the lesion was observed throughout the three weeks' observation period and was more striking than the slowness and laxity of movement although seen only when the macaque did not follow his per-

over-actions might be interpreted as "hyperpathia" Tactile stimuli were recognized after eight months, but were not localized, and food particles brought into contact were apparently not appreciated as such since it did not usually grasp them on the side opposite the lesion Another consistent indication of sensory loss, more in the nature of a deficit in proprioception, was the failure to correct bizarre positions of the limbs until he had noted them visually

B Hopping and placing reactions The hopping reaction was normal after a month Proprioceptive placing was present after this time also, but a greater angle of displacement at the thigh and shoulder was necessary than in the normal side and the execution was slower and at times ataxic, the foot striking the table too low The angle of displacement necessary to initiate movement gradually became less during the first two months, then became fixed, but never decreased to the normal Although tactile placing returned in nine months, its initiation was delayed This finding may have been due to the general slowness of motor performance and/or to an increased threshold and a slowness in perception of the stimulus Occasionally the movement was ataxic

Ablation of area 3 and areas 1-2 Seven animals, three from area 3, P4, P7, P11, four from areas 1-2, P8, P9, P14, P17

"ACUTE"

I PERFORMANCE DURING 1ST TO 14TH DAYS Animals with removal of area 3, and those with removal of areas 1-2 were similar and will be considered together The disturbances were at all times more serious following areas 1-2 ablations than area 3 ablations although their quality and distribution were similar The two groups of animals in the early post-operative period were quite similar to, but not as severely handicapped as, those with the largest ablations described above They were loathe to use the affected hand, though they could when necessary Ataxia accompanied movements not followed visually Associated movements of extension and protraction of the arms frequently mirrored similar movements of the normal limbs Hypotonia was present and its distribution resembled that described for the larger ablations Tendon reflexes were characterized by an increased threshold, they were slow in execution, and their scope was greater than those of the normal Superficial reflexes were unaltered Response to pin and light touch was lost and there was a loss of placing, in response to both tactile and proprioceptive stimuli, hopping was absent

II PERFORMANCE DURING 14TH TO 21ST DAYS A remarkable improvement was noted by the end of two weeks No lack of desire to use the affected hand existed There was, however, a slowness in its movements Ataxia was less The affected foot was not lifted as high in walking on a surface and was not as awkward in walking the rope as it had been Hypotonia and alteration of reflexes as to increased threshold and excursion persisted Response to light touch was absent, and an object placed carefully in the hand was slowly grasped Response to pain was present but slower than normal, and localization of painful stimuli was impossible Placing in response to tactile stimuli failed, placing and hopping in response to proprioceptive stimuli had returned but the angle of displacement required at the thigh and shoulder was greater than the normal At the time of sacrifice the findings were similar

"CHRONIC"

I MOTOR PERFORMANCE After twelve months the "chronic" animal (P11) with ablation of area 3 resembled the one (P17) with removal of areas 1-2 Both were capable of quick, purposeful movements without ataxia when checked by vision The animal with areas 1-2 removed, for example, caught with its hand small bits of paper thrown at it, and rarely missed It used either hand or foot at will in motor activity There was no ataxia when movement was watched In stair climbing and rope walking, however, where each movement was not watched there was, after a year, some over-stepping of steps and searching for the rope

II CONDITION OF "TONE", REFLEXES Although hypotonia was similarly distributed in each of these macaques it was greater in the 1-2 animal The tendon reflexes in both of these macaques were pendular after a year and not as slow as they were in the first few post-operative months Again the area 3 animal was less affected than the areas 1-2 animal Superficial reflexes were equally active in each

to the point stimulated. Impairment in response to pin prick was greater over the extremities than over the trunk, and in the extremities more so in the palm and sole. A lowering of threshold of the response was noted by the time of sacrifice of the "acute" animals. Response to tactile stimuli was impaired to greater degree than to pin prick, for light touch evoked no response, whatever. Further evidence that tactile stimuli (among others) were imperfectly received was the failure of the animals to recognize and grasp food particles such as rice grains, nuts, and raisins, placed lightly in the affected palm. Similarly, there was a failure to grasp any object brought into gentle contact with the sole or palm.

When the animals were at ease and relaxed, and paying attention to some object in the room, the affected lower limb placed in a bizarre position was maintained until they caught sight of that extremity. Immediately, the abnormal posture was changed to the normal.

B Hopping and placing reactions Hopping and placing reactions, proprioceptive and tactile, were found useful in evaluating sensory loss. No response was obtained in the hopping, proprioceptive or tactile placing tests in the early post-operative period, but usually within the three weeks' observation period, response to hopping and proprioceptive placing had returned. Both were slower than the hopping and placing performed by the normal limbs, because greater angles of displacement of the thigh and of the upper arm were required for the affected limbs than for the normal extremities. The movement itself was more slowly executed than that performed by the normal ones. The existence of hypotonia caused ataxia in the placing responses, errors in measurement and in direction were noted. Tactile placing was absent in these animals at the time they were killed. This was not a motor deficit because the affected foot frequently followed the normal limb in placing in response to tactile stimuli.

"CHRONIC"

I MOTOR PERFORMANCE *A Spontaneous use of skeletal muscles* The one "chronic" animal (P19) from which areas 1, 2, 5, and 7 had been ablated showed gradual improvement during the year's observation. Preference was given to the normal limbs when motor activity had to be carried out quickly, all four extremities were used interchangeably when angry. It scratched with the two hands equally well and with similar movements. The quiet movement of the affected limbs was slower than that of the normal ones. No progression of flexion and adduction occurred in the muscles of the fingers. A variety of decomposition of movement was used, namely, a slow flexion, pause, slow adduction, or several movements of the same type, then several of the second type. Alternate movements toward and from the object were performed at the shoulder, and alternate extension and flexion at the elbow and wrist were observed as the object was approached. Slowness of movement and looseness of fixation of the joints were noticeable.

B Ataxia Ataxia remained, but was less noticeable than in the early post-operative period. The animal continued to under- or over-reach the object sought. It continued to strike the affected foot against the step in stair climbing, because it kept the toes extended loosely and the ankle partially ventroflexed although flexion of the toes and dorsiflexion of the ankle was possible. No associated movements were observed after the first month.

II CONDITION OF "TONE" IN SKELETAL MUSCLES *A Resistance to passive movement, reflexes* Hypotonia persisted in the muscles contralateral to the lesion and was equal in quality and distribution to that seen in "acute" preparations. The initial drooping of the shoulder and loose extension in all the joints of the lower limb remained unchanged. The tendon reflexes were always characterized by increase in threshold and the excursion though great was executed slowly by the arm and leg opposite the lesion, differences in threshold and excursion after a year, however, between them and those of the normal side were not as marked as at first.

B Atrophy The drooping shoulder and the dragging of the toes may have been due in part to atrophy. Though no measurements or weights of the muscles were made there was an apparent difference in size between the limbs of the two sides. This was more noticeable in the hands and feet, the affected hand and foot appeared thinner than the normal, and the affected fingers and toes were more tapering and slender.

III SENSORY LOSS *A Response to varied stimuli* The response to pin prick was interesting, particularly since the animal was very friendly and very easy to examine. When pricked lightly anywhere on the affected side it snarled, but it remained impassive when pricked on the normal side. It was indifferent to efforts to elicit a grasp in the normal hand, by placing a pencil or a finger in its palm, the affected hand was withdrawn. These

I **MOTOR PERFORMANCE, LOWER LIMB** The sensory deficit was greater in the lower limb than in the upper in these animals after four and twelve months respectively. The foot contralateral to the lesion was always used secondarily, the hands interchangeably. Finger and toe movements were well executed. Even after a year, ataxia accompanied movement of the leg in stair-climbing and in rope-walking, when the animal was not paying attention. The lower limb was usually insufficiently abducted at the hip, and insufficiently extended or flexed at the knee and ankle to avoid striking the stairs by the dorsum of the foot. The rope was missed by the foot and toes in the first attempt at grasping for it. A slight associated protraction or flexion of the affected thigh occasionally accompanied stretching or reaching movements of the ipsilateral arm in P13.

II **CONDITION OF "TONE", REFLEXES** Hypotonia in the affected limbs was more noticeable in the leg than in the arm, and more posturing was observed in the lower limb than in the upper, though this was also slight in the leg. Hypotonia was greater in the abductors, the hamstrings, and the dorsiflexors of the foot than in the other muscles of the leg. The tendon reflexes remained for the duration somewhat pendular in the leg, in the arm they approached the normal. The superficial reflexes were normal.

III **SENSORY LOSS** Pin prick was quickly recognized after the first three months. Localization of prick was never observed. Recognition of tactile stimuli was present after three months, but food particles were not appreciated when brought into contact with the foot.

The hopping and proprioceptive placing reactions had returned in the leg as well as in the arm after two months, but proprioceptive placing even after a year required a larger displacement at the thigh in the leg contralateral to the lesion. The response in the arm appeared normal. Tactile placing also returned in the leg as well as in the arm after three months, and the initiation and execution of the response was slower than in the normal limbs. This was particularly true for the leg.

Ablations of area 7 Three animals were used P5, P12, T3

"ACUTE"

I **MOTOR PERFORMANCE, UPPER LIMB** The macaques from which area 7 had been removed were less handicapped than those in which area 5, or area 3, or areas 1 and 2 had been ablated. Ablation of area 7 produced more disturbance in the contralateral upper extremity than in the lower. Movements of the fingers and toes were well executed when they were watched, but noticeable awkwardness and under- or over-shooting appeared when visual aid was not given. The normal hand and arm usually led in motor performance and were favored if they alone would suffice, their movements were carried out more quickly, while the affected arm and hand appeared to grope unless watched closely. No associated movements were observed.

II **CONDITION OF "TONE", REFLEXES** Hypotonia was present early in both arm and leg in these macaques, but grew less apparent during the observation period, particularly in the lower limb. The "paretic posture" was more evident in the upper limb in which there was slight drooping of the shoulder, loose flexion of the elbow, and extension of wrist and fingers at rest. Little difference in the posture of rest of the two upper limbs was seen, however, by the end of the three weeks' observation period. Resistance to palpation and to passive movement remained less in the upper extremity opposite the ablation. The reflexes were characterized by increased threshold, wide excursion, and slow performance during the first two weeks particularly in the arm, after this they resembled those of the normal limb.

III **SENSORY LOSS** Appreciation of pain and tactile stimuli was not observed over the upper limb and hand, during the first post-operative three weeks, although these stimuli were appreciated during this time over the lower limb and trunk, as judged by the motor reaction to them, they were not localized. Neither appreciation of food particles by contact nor their localization was observed during the three weeks' examination period. After two weeks proprioceptive placing and hopping responses returned but required for their initiation a stimulus of greater displacement than did the normal extremities. Tactile placing appeared in the leg by the end of the three weeks' period though it was definitely slower, for the threshold was increased and the initiation delayed.

III **SENSORY LOSS** Both animals were slow to respond to pin prick and usually made no effort to localize it. Response to tactile stimuli as shown by grasp was usually present at the end of the 9th or 10th month. Occasionally there was no response. This was particularly true of the monkey with areas 1-2 removed. Both animals, however, were quick to rectify without visual aid and unusual postures passively produced.

Hopping and proprioceptive placing were always present after two months. The necessary angle of displacement decreased gradually in the affected limbs in the early post-operative months, but never became equal to that of the normal side. Tactile placing was present at times after nine months in the area 3 monkey but longer duration of stimulus was required than normal and execution of response was slow and often awkward, tactile placing was absent permanently in the 1-2 animal.

Area 5 ablation Four animals were used P3, P10, P13, P18

"ACUTE"

I **CONDITION OF UPPER EXTREMITY** These macaques were similar post-operatively, though three had left sided operations, and one a right sided. Their general behavior was similar to that of the animals with the large ablations, but the leg appeared to be more impaired than the arm. Changes in the upper extremity were present, but were milder than those observed in the macaques with ablations of area 3 or of areas 1-2.

The affected hands were used as readily as the normal when feeding or scratching or in reaching for desired objects which could be followed with the eyes. Ataxia when not watching the movements performed was the most striking change in the affected arm and hand in these monkeys. When casually reaching for an object which was not particularly desired the movements were ataxic. The affected arm and hand nearly always overshot the mark in "climbing" a suspended cord, for example, and showed ataxia in grasping the cage bars unless corrected by sight. No associated movements were observed in the arm. Hypotonia was slight in the arm, and the threshold of tendon reflexes was only slightly increased. The grasp in response to light contact was slow in the hand. Recognition of painful and of tactile stimuli was present but their localization was poor. Proprioceptive placing returned within three weeks—but tactile placing when present was slow in initiation as well as in execution.

II **CONDITION OF LOWER EXTREMITY** A *Spontaneous use* Loathness to use the affected lower extremity was striking, and the manner of its use was not normal. The leg was adducted in walking on a flat surface, and was lifted much higher than the normal, and the hallux struck or rubbed against the normal leg, in stair-climbing, the foot often brushed against the next highest step because the degree of flexion of the ankle and toes was insufficient. Ataxia in rope-walking was marked, for the leg was either too greatly abducted or adducted. Associated movements of the affected foot were observed only during the early post-operative period. Simple protraction of the leg in an extended-adducted position accompanied the extension of the normal extremities in reaching for a desired object.

B *Condition of "tone", reflexes* Hypotonia was more marked in the abductors of the thigh, the hamstrings and tibialis anterior than in the remaining muscles of the lower extremity, but was never as great as that found after ablations involving the postcentral gyrus. The tendon reflexes in the leg had higher thresholds than those in the arms. The arc of their excursion in the leg was greater and the time of their total execution was longer than in the homologous arm.

C *Sensory loss* Pin prick was recognized but slight tactile contact with the sole produced either a deliberate flexion of the toes or none at all. Localization of touch and painful stimuli over the lower limb was inaccurate when judged by scratching or grasping response of normal extremities. A failure to recognize or appreciate food particles as such was present. Tactile placing by the lower extremity was absent throughout the three weeks' period, proprioceptive placing and hopping were present after two weeks, but required greater angles of displacement of the thigh than did the normal leg.

"CHRONIC"

Two animals were used P10 and P13. One accidentally hanged itself in the leash 110 days post-operatively, the other was sacrificed 370 days post-operatively. Both presented similar early signs though the monkey which was killed after a year had made more improvement.

distribution to leg and arm respectively. Alteration of tendon jerks was permanent after all ablations, and of greater degree after ablations involving the postcentral gyrus. Superficial reflexes remained normal.

Response to pain stimuli was present but delayed after three weeks in all animals. Tactile stimuli were recognized after two months by area 5 and area 7 animals, after nine months by area 3, areas 1-2, and areas 1-2, 5, 7 animals. Localization of pain and tactile stimuli was deficient in all for a year. Response to pinprick was exaggerated after six to eight months in the animal with ablation of all the parietal lobe except area 3. Food particles were not appreciated as such at any time after all ablations. In the area 5 animal all the difficulties were more noticeable in the lower limb, in the area 7 animals in the upper. Slight wasting of muscles in the contralateral limbs was noted in the one animal with all areas except 3 removed. No deficiency of any type was observed in the ipsilateral limbs.

Tactile and proprioceptive placing and hopping reactions were absent in all animals in the early post-operative period. Hopping reactions returned in all after the early months, and proprioceptive placing returned in all. The movement, however, was slower, required a greater stimulus of displacement and frequently was ataxic after postcentral ablations. Tactile placing was more severely impaired than proprioceptive placing, after areas 1-2 ablations, it never returned, after area 3 ablations, tactile placing returned after two to three months, but was still slower than normal both in initiation and execution and was frequently awkward, following ablations of area 5 and area 7, similar delay was observed especially in the leg and arm respectively, and frequently the animal did not place in the direction from which the stimulus came.

DISCUSSION

A certain localization of function in the parietal lobe is suggested by an analysis of the results described above, correlation of function with known anatomical connections can also be made. No paresis of individual muscle movement after parietal lesions is suggested by the relative paucity of corticospinal fibers from that lobe, and by the type of movement elicited by electrical stimulation over the parietal areas. The loathness to move could be accounted for by the loss of incoming stimuli, plus the inaccuracies of movement resulting therefrom.

Hypotonia was consistently present after parietal lesions, being more widely distributed and of greater degree after the large ablations. Brodmann (3) and Ferraro and Barrera (6) described hypotonia after postcentral lesions, but gave no distribution. Kennard and Kessler (14) described persistent "diminished resistance to passive movement" after all their parietal lesions, tendon reflexes were only temporarily diminished. Hypotonia, in the animals in the present work, was most noticeable in the upper limb in the elevators and abductors of the shoulder, the external rotators of the arm, the extensors of the forearm, the flexors of the wrist and fingers, and the

"CHRONIC"

I MOTOR PERFORMANCE, CONDITION OF "TONE", REFLEXES Two animals (P12, T3) with ablations of area 7 were observed for a year, and nine months, respectively. The upper limb was more impaired than the lower and with this exception these monkeys were similar in their general abilities to the "chronic" area 5 animals. Slowness of movement continued in the upper limb throughout the year and ataxia accompanied movement not checked by vision. Hypotonia was only slightly noticeable in the elevators of the shoulder, the extensors of the arm, the flexors of the wrist and fingers, after the first three weeks post-operatively, and parietic posturing was not observed. All tendon reflexes remained pendular. Their threshold was raised, and they were slow in execution. A slightly stronger stimulus was required to obtain a response in the upper limb than in the lower, and the execution of the movement was slower in the arm than in the leg.

II SENSORY LOSS Response to pin prick was similar on the two sides after a month, but effort to localize it was confined with rare exceptions to the normal extremities. Objects placed lightly in contact with the palm were grasped, but food particles placed in the hand contralateral to the ablation were not recognized as such without visual aid. Proprioceptive placing and hopping were normal after the first month. Tactile placing returned after two months, but was slowed in initiation and execution, and the animal did not always direct the extremity toward the locus of the stimulus.

Summary of functional deficits, "acute" and "chronic" Ablations from the individual areas of the parietal lobe were followed immediately by certain signs the sum of which compared well with, but were not equivalent to, the whole following removal of areas 1, 2, 5, and 7 together. Ablations of the postcentral areas produced changes in both the contralateral arm and leg, of area 5, greater in the leg than the arm, of area 7, greater in the arm than in the leg. Ablation of area 5 or of area 7 did not result in as severe disturbance in either the leg or the arm, respectively, as did postcentral lesions.

Paralysis was not observed after any ablation, rather, slowness in the initiation and execution of movement, and motor performance continued to be slow except under emotional stress. Following all ablations, ataxia characterized movement which the animal did not visually control but it seemed never to occur when the animals paid strict visual attention. It was present in both extremities following removal of area 3, or areas 1-2, the lower limb was more awkward after area 5 ablations, the upper, after area 7 removals. Involuntary associated movements were more often observed after postcentral ablation, and were present only for the first post-operative month except in one area 5 animal, in which they were seen occasionally for a year.

Hypotonia, as demonstrated by decreased resistance to palpation and to passive movement, was present after all lesions. After ablation of areas 3 or 1-2 the distribution and the quality of this hypotonia were similar in the arm and leg, but following ablation of area 5, hypotonia was greater in the leg than in the arm and after removal of area 7 it was greater in the upper limb. The hypotonia was persistent in all "chronic" animals, though more noticeable in the animal with the large ablation, and in those with destruction involving the postcentral gyrus, than in those with extirpation of area 5 or of area 7. The tendon reflexes were characterized by an increase in excursion, a raised threshold and lack of normal quickness in response. After area 5 or area 7 ablations the hypotonic tendon reflexes had a differential

specific motor neurons. In such a mechanism promoting sensory attention and efficient motor neuron discharge, deficiencies of "tone" are counteracted. Hypotonia present after parietal ablation, as well as deficient integration of incoming peripheral sensory stimuli, were together accountable for the slowness of movement seen in these animals.

The alteration of the tendon reflexes can be similarly explained. The loss of proprioceptive integration and subsequent deficiency of sensory attention, resulting in raised threshold of peripheral inhibitory and activating proprioceptive endings, serves to deprive the ventral motor neurons of prompt and efficient activating stimuli. The alteration of proprioceptive activation and inhibition becomes slowed and disorderly as a result of inadequate central integration and sensitization.

The sensory losses resulting from parietal ablation can be explained more adequately. The losses found, moreover, would be expected on the basis of anatomical information. According to Walker (26), the nuclei of termination of the medial lemniscus and the spinothalamic tracts project to cortical areas 3, 1, and perhaps 2. These areas in turn send fibers to these nuclei, *ventralis posteromedialis* and *ventralis posterolateralis*, transmitting thence corticofugal sensitizing stimuli. After removal of area 3, and of areas 1-2 appreciation of pain was deficient for three weeks, appreciation of tactile stimuli, for nine months. Localization and discrimination of these stimuli were permanently deficient. Cutaneous and deep sensibility were disturbed over the contralateral side of the body equally after ablations of postcentral areas and no ipsilateral losses were observed. The loss of appreciation of tactile stimuli resulting from ablation of the postcentral gyrus coincides with the results of Woolsey, Marshall and Bard (29) who measured cortical potentials arising as a result of tactile stimulation peripherally.

Areas 5 and 7 receive few fibers from the thalamus and these are chiefly from the *nucleus lateralis posterior* (26). These areas project to that nucleus, as well as to the nuclei *ventralis posterolateralis* and *posteromedialis*. To Walker the *nucleus lateralis* is a higher level of integration than the *ventral nuclei*, and receives fibers from them, areas 5 and 7 are to him higher levels of cortical sensory integration, receiving from *nucleus lateralis posterior* a summary of *ventral nuclei* reports. Topographical arrangements were not considered by him in these areas. The relative paucity of fibers exchanged by these areas and the thalamus, as compared to those exchanged by the postcentral gyrus and the thalamus, would, however, lead one to expect fewer manifest simple sensory losses as a result of removal of either. Appreciation of tactile and pain stimuli was in fact only temporarily altered, their localization was more permanently disturbed. The topographical differences noted in the results of the two ablations might be inferred somewhat by the projection of parieto-spinal fibers from 7 only to cervical levels, from 5, to lumbar as well. Rothmann (20) reported more disturbance in the upper limb as a result of removal of the "supramarginal" gyrus (area 7).

Slowness of movement, ataxia, and the failure to correct abnormal pos-

adductors of the fingers. The proximal parts of the limb were more hypotonic than the distal. In the leg, the flexors and extensors of the thigh, and the flexors and extensors of the leg were about equally affected, the external rotators of the thigh, the dorsiflexors (tibialis anterior especially) of the foot, and the extensors and adductors of the toes were more hypotonic than their antagonists. The variation noted in the topographical distribution of hypotonia following the different parietal lesions might be suggested by the distribution of the parietospinal fibers. These fibers were most numerous from areas 3-1-2 and passed as far caudally as lumbar levels, those from area 5 were more numerous than those from area 7, and while all those from 7 terminated at cervical levels, some of those from 5 passed to lumbar levels.

Hypotonia following the larger parietal ablation resembled for the most part in its distribution that described by Tower (24) to follow pyramid section. It seems probable then that after pyramidal section, it is the severance of parietospinal fibers which is at least in some measure responsible for the development of the hypotonia. The mechanisms through which it develops are difficult to ascertain, even though hypotonia clinically is associated with lesions of the sensory path at dorsal root, cord, and thalamic levels, as well as at cortical levels.

Muscle "tone" is usually interpreted as a product of the myotactic reflexes. In cats, Lloyd (16) has found these latter to involve two neurons only, the proprioceptive and the large ventral horn cell. This ventral neuron is directly influenced by descending extrapyramidal tracts and only indirectly by pyramidal fibers. He considered it likely that the proprioceptive fiber terminating about this ventral neuron sent collaterals to terminate about certain neurons in the posterior gray, especially those giving rise to spinocerebellar tracts. It seems reasonable to suggest that it is the loss of integration of these ascending proprioceptive impulses at thalamic and cortical levels, and consequent deficient or altered efferent discharges therefrom, that indirectly is responsible for alterations of muscle "tone." "Tone"-giving impulses traveling via corticospinal and parietospinal fibers and arising as a result of integration of incoming proprioceptive impulses are thus possibly essential for normal maintenance and distributions of "tone." Corticospinal (pyramidal) fibers in Lloyd's experiments were found to activate the posterior gray neurons directly, and these in turn set up activity in the anterior motor neurons. Parietospinal fibers have been considered in the present work to have terminations in the anterior portion of the posterior gray, and it has been suggested that they contribute an energizing or sensitizing influence on the sensory neurons at cord level. There can be thus two balancing mechanisms descending into the spinal neurons contributing influences on maintenance of "tone" in skeletal muscle. Certain extrapyramidal fibers activate (or inhibit) motor neurons directly and prevent excesses of "tone"; pyramidal and parietospinal fibers activate and sensitize to incoming peripheral stimuli, respectively, sensory neurons (or at least posterior gray neurons), making it possible for them in turn to activate selectively

with ablation of all areas except 3 is rather interesting. Why other animals did not show it or why this one did is unknown. The large amount of tissue removed from the parietal area in this monkey, plus the adequate time for complete degeneration of severed tracts, may be factors concerned in the appearance of this phenomenon. The occurrence of "hyperpathia" in humans has been reported in lesions limited to parietal cortical tissue, and has also been described in thalamic, cord, and bulbar lesions (4). Head and Holmes (13) attributed "hyperpathia" to a loss of cortical inhibition on the thalamus while Wilson (27) felt it resulted from irritation to a partially damaged spinothalamic system at any level. Can it be interruption of parietothalamic, parietopontine, and parietospinal fibers with the subsequent loss of their sensitizing, energizing, modulating or inhibitory influence, that results in "hyperpathia"? Foerster (7) apparently had such a scheme in mind when he postulated descending fibers in the lateral funiculus of the cord that exert an inhibitory influence on spinothalamic fibers.

Muscular atrophy following unilateral parietal lesions in humans has been reported by Guthrie (11), Head (12), and Winkelman and Silverstein (28) among others. Kennard and Kessler (14) reported bilateral muscle atrophy in monkeys following removal of all parietal tissue bilaterally. The one "chronic" animal with the destruction of all unilateral parietal tissue except area 3 in the present work showed a thinness and tapering of the hand, foot, and digits contralateral to the lesion. Though no measurements were made, this observation seemed quite definite. The explanation of atrophy occurring in parietal lesion is not easy. Vasomotor and trophic changes possibly responsible were not studied in this present work, and perhaps are only found after gross parietal destruction.

The nature of the ablations and the subsequent results preclude any statements as to the sole representation of any one sensory function in any one area. Localization of function in the parietal lobe, however, can be said to be somewhat as follows on the basis of these studies. Cutaneous sensibility is particularly represented in the postcentral gyrus. Discriminative ability is more lastingly impaired than mere recognition of stimuli, however, and depends on integrations involving all parietal areas. Proprioceptive stimuli, necessary for coordinated movement, regulation, and distribution of normal amounts of tone, and supraspinal influence of tendon jerks, terminate also in all parietal tissue, but apparently end also in other cerebral areas, possibly the precentral. There seems to be a topographical variation in sensory localization in the parietal cortex, all skin area finds a postcentral locus, while in areas 5 and 7 the limbs seem more specifically represented. This topographical arrangement is somewhat confirmed by the cortical map of parietal localization compiled by Dusser de Barenne (5) on the basis of his strychninization of the monkey's cortex. It is seen from his map that area 5 is in the so-called leg zone, area 7, more in the arm zone. Ample pathways exist for transmission of parietal cortex influences, and these are associative and corti-

tures, after the larger ablations, particularly, can be accounted for through the lack of cortical integration of, or the actual loss of, many of the proprioceptive stimuli arising as a result of movement, actively or passively initiated. All of these abnormalities of movement except slowness were corrected under emotional stress or by close visual cooperation. Although even when observed by the animal, this characteristic of movement was often present especially after the larger ablations. Proprioceptive impulses, terminating precentrally after a synapse in a thalamic nucleus (*lateralis ventralis*), alone and without visual aid were insufficient to provide adequate information to the motor mechanisms for smooth performance. Their inadequacy is even more noticeable in responses involving tactile and proprioceptive discrimination and localization.

The hopping and placing reactions as described by Bard are thought to involve cortical arcs of neural activity. Placing reactions performed without the aid of vision are divided into two groups: those elicitable by light contact of the limb with a surface and those requiring angular (or proprioceptive) displacement for a response. Hopping reactions and proprioceptive placing have been considered to depend ultimately on the integrity of the precentral gyrus, while tactile placing depends on postcentral tissue as well. (1) All these reactions were absent after all ablations for the first few post-operative days. Hopping and proprioceptive responses were usually obtained after all ablations within the early post-operative weeks, but larger displacements were required, and this requisite persisted throughout a year following an ablation of area 5. Tactile placing was slower to return in all animals and after ablations involving postcentral areas never returned to normal. It would seem that though all the parietal areas take part in the normal response in tactile placing, the postcentral areas are more essential. The parietal areas are not essential, however, for the performance of normal hopping and proprioceptive placing reactions, and the fact that these reactions return so quickly after the larger ablation of parietal tissue (though this spared area 3) can be considered further evidence of an extra-parietal cortical termination for proprioceptive stimuli. Similarly, the fact that tactile placing is so long disturbed after a parietal ablation, especially one involving postcentral areas, seems evidence for a less important, if any, extra-parietal locus for these reactions. Bard (1) found that hopping reactions were only temporarily affected by postcentral ablation, and that even after removal of all cortex except that of the frontal lobe, hopping reactions soon returned to normal and proprioceptive placing could also be "elicited." Kennard and Kessler (14), however, were of the opinion that proprioception is represented in postcentral regions since slight, though permanent alteration in response appeared after small parietal lesions, and further since proprioception was permanently affected if all the parietal lobe was removed from both hemispheres.

The apparent existence of "hyperpathia" in the one "chronic" animal

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cifugal Thalamus, midbrain, pontine nuclei and cerebellum, as well as cord neurons, come under its sphere of influence, whether this be inhibitory, sensitizing, modulating, or energizing, and thus there is made possible a more harmonious, better coordinated and more efficient sensory mechanism which is directly responsible for more effective motor discharge

SUMMARY

1 Removal of area 3, areas 1-2, area 5, and area 7 individually, or of areas 1-2, 5, and 7 in combination, from the parietal lobe of the macaque, did not result in paralysis. A loathness for movement was present.

2 Removal of area 3 or of areas 1-2 affected the contralateral arm and leg equally, removal of area 5 affected the leg particularly and of area 7, the arm particularly. The disturbances are described below. Such a differential localization of them might be inferred from the distribution of the parieto-spinal fibers which have also been described.

3 Hypotonia was consistently found for as long as a year, but was not present equally in all contralateral muscles, the proximal muscles being more hypotonic. Of those affected in the upper limb, the elevators and abductors of the shoulder, the external rotators of the arm, the extensors of the forearm, the flexors of the wrist and fingers, and the adductors of the fingers were most hypotonic. In the leg, the flexors and extensors of the thigh were about equally affected, the external rotators of the thigh, the dorsiflexors of the foot, and the extensors and adductors of the toes were more hypotonic than their antagonists. The mechanisms underlying this hypotonia have been discussed. Hypotonia probably also accounted for "the parietic posture" at rest and indirectly for ataxia and slowness of movement. Ataxia was not present when the macaques controlled movement by vision.

4 Tendon reflexes were permanently altered by an increase in threshold, a slowness of execution, and an increase of excursion.

5 Appreciation of tactile and painful stimuli was impaired initially after all ablations and for nine months after postcentral lesions. Localization of these stimuli was persistently impossible after all ablations.

6 Proprioceptive placing and hopping, and tactile placing were absent immediately after all ablations, the two former reappeared after three weeks but larger displacements were required. Tactile placing never returned to normal after postcentral lesions, and though it returned after ablation of area 5 or area 7 there was a slowness of response and some uncertainty in its direction. Postcentral gyri appeared essential for tactile placing and shared with extra-parietal cortex the mechanisms involving proprioceptive placing and hopping.

7 The postcentral gyrus appeared essential for the recognition of painful and tactile stimuli, all parietal areas were necessary for the localization and discrimination of these stimuli.

8 Muscle atrophy and hyperpathia were observed in one animal as chronic results of removal of areas 1-2, 5, and 7 together.

INHIBITION OF ACTIVITY IN SINGLE AUDITORY NERVE FIBERS BY ACOUSTIC STIMULATION*

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INTRODUCTION

THE CHARACTERISTIC responses of single auditory nerve fibers have already been reported (5), and may be summarized as follows. When acoustic stimulation is delivered to the ear of a cat, each auditory fiber is most sensitive at a specific frequency and increases its rate of discharge with increasing intensity of stimulation. Also the louder tones excite more extensive areas of the basilar membrane. The evidence appears to support a place theory of action of the mammalian cochlea.

In the present report it will be shown both that spontaneous activity in auditory fibers may be abolished when appropriate sounds are presented to the ear, and that inhibition may result when a second tone is sounded in the presence of a first tone which initiates nerve impulses when sounded alone.

A simple place theory assumes that a given pure tone sets in vibration a restricted region of the basilar membrane and has no effect upon others. One would predict, therefore, that a nerve fiber activated by a pure tone will be uninfluenced by a second pure tone so long as the two tones are sufficiently far apart in frequency. As the second tone approaches the first, however, the two excited regions of the basilar membrane begin to overlap, and the fiber might under these conditions be expected to discharge more vigorously. When the tones are of the same frequency (and in phase), the fiber would presumably be stimulated most effectively. The results of our experiments with two tones are not wholly in accord with the simple picture just outlined. Instead, there is marked inhibitory interaction between tones even many octaves apart. And this inhibitory interaction is most simply explained by assuming the presence of a specific neural inhibitory mechanism within the cochlea.

METHOD

The use of Ringer-filled micropipettes (3–5 μ) as electrodes for isolating the electrical activity of single auditory nerve fibers, and the methods for producing single pure tones, have been described (5). To present two pure tones simultaneously, a second oscillator† and attenuator were introduced in parallel with the G.R. 713B oscillator, and the outputs of both led to the speaker transformer. The experimenter could thus generate tones with

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‡ Either a Clough-Brengle model OBR, or, more often, a Hewlett-Packard model

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The insert in Fig 1 shows the result of stimulation of a fiber (9400 c p s -35 db)* with a tone of its characteristic frequency at various intensities. Spontaneous activity is clearly reduced by certain tones, and the degree to which each tone is effective depends upon its intensity, as shown in the accompanying graph (Fig 1). Spontaneous activity is almost completely obliterated at -44 db (below 2 volts from oscillator), while some inhibitory

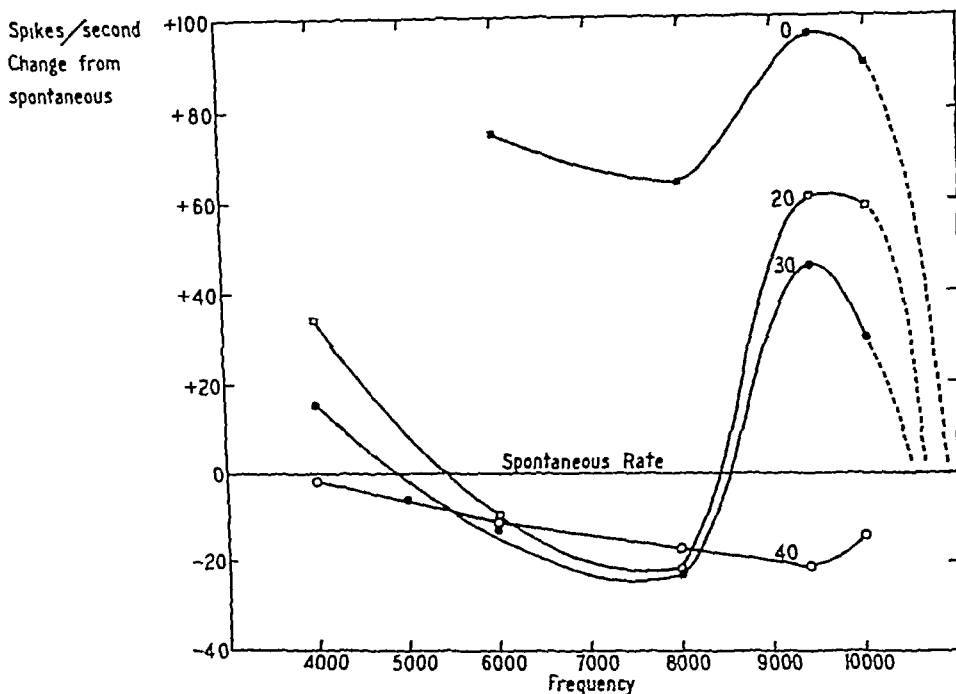


FIG 2 Iso-intensity contours showing inhibition of spontaneous activity by acoustic stimulation. Numbers on the contour lines indicate the intensity level (in db below 2 volts from oscillator) at which the rate of nerve discharge was determined as a function of frequency. Data from same fiber as Fig 1.

effect can be seen between -80 and -36 db. At intensities above -34 db the fiber responds to increase of intensity by accelerating its rate of discharge, as would normally be expected from stimulating the end-organ with sound (5).

Spontaneous activity can be reduced or abolished by frequencies other than the characteristic frequency. Figure 2 shows iso-intensity contours for the same fiber (9400 c p s, -35 db). At an intensity level of -40 db, the

* The numbers in parentheses give the "characteristic frequency" (9400 c p s) and "minimal intensity" (35 db below 2 volts from oscillator) for the fiber. It has been previously established (5) that at a certain "minimal intensity level" the adequate stimulus is a small band of frequencies, the "characteristic frequency," and at lower intensity levels no frequency excites the fiber.

either of these oscillators alone, or with both together. Intensities were measured as decibels below 2 volts from the G R 713B oscillator. With our sound system the threshold for the human ear at 2000 c p s is approximately -100 db. Readings obtained with the second oscillator system were converted to equivalent readings on the G R 713B system, so that the data are all directly comparable with those presented earlier.

Two neon bulbs at the edge of the tube-face signalled the duration of presentation of the sounds used as stimuli. On the records the lower of the two white lines is caused by the neon bulb in the G R 713B circuit. The upper neon bulb was manually operated and indicates only approximately the period of stimulation.

It should be stressed that the results described here are independent of the method used to produce the sounds. Human voices substituted for the oscillator systems are just as effective.

RESULTS

1 *Inhibition of spontaneous activity by pure tones.* About half of the auditory-nerve fibers in cats discharge at a slow steady rate ("spontaneous activ-

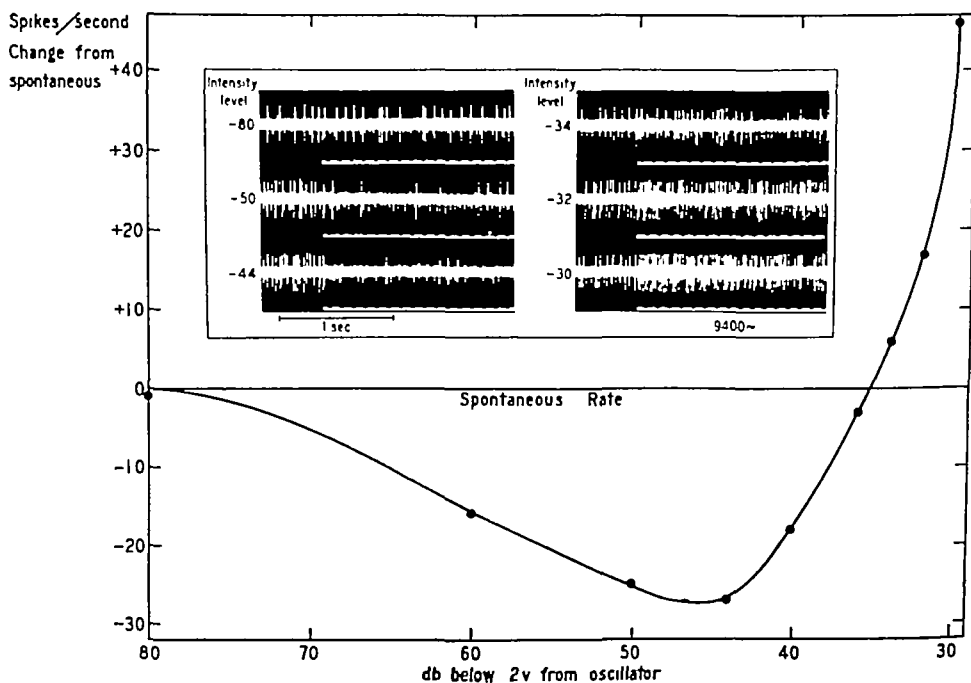


FIG 1 Inhibition of spontaneous activity by acoustic stimulation. Insert shows some of the records used in constructing the graph. White line below record indicates onset and duration of the 9400 c p s tone at the intensity level indicated. Points were determined by counting the spikes during the first second after the onset of the tone, and subtracting from this the number in the preceding second. This fiber has a characteristic frequency of 9400 c p s and a minimal intensity of -35 db.

ity") even though precautions have been taken to eliminate ambient noise (5). Some of the fibers exhibiting this behavior become markedly less active when noises, the human voice, or pure tones are presented to the ear.

inhibitory effect was marked at the characteristic frequency. The more usual relation is illustrated in Fig 3, where both "response area" and "inhibitory area" are shown for two fibers. The response area, as previously defined (5), comprises all tones which increase activity, while the term *inhibitory area* will be used hereafter to designate those tones which depress activity in the

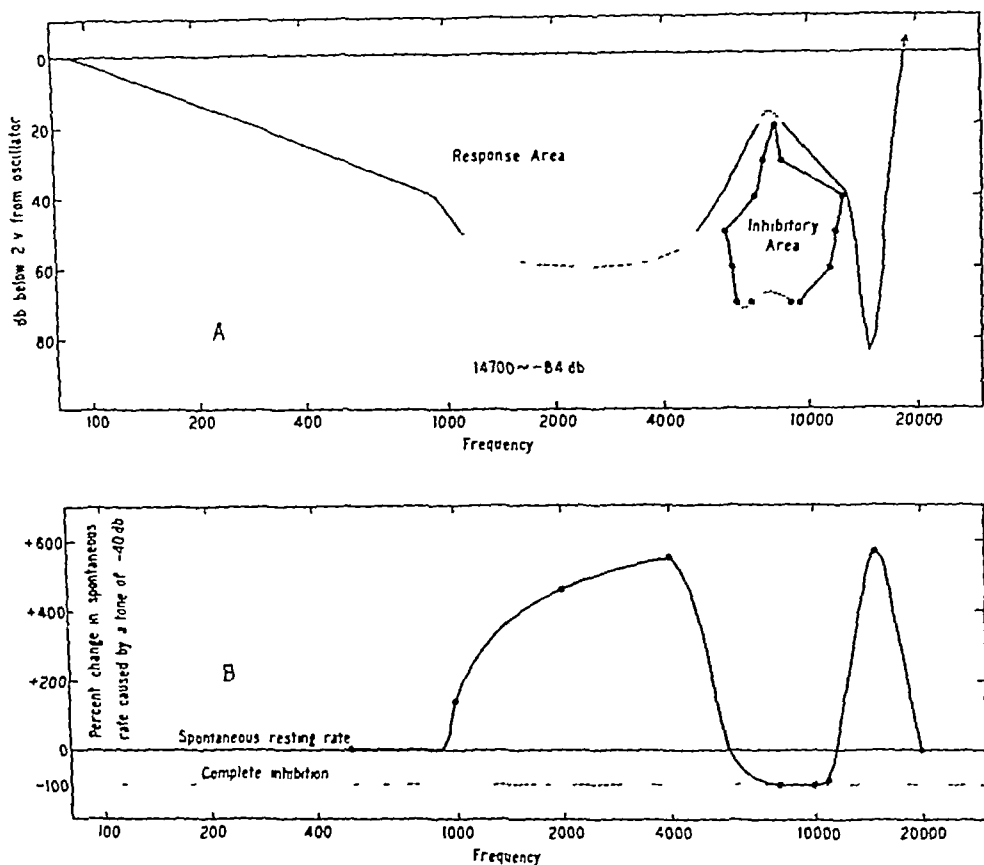


Fig 4 Inhibition of spontaneous discharge by acoustic stimulation. A All tones within the response area caused the single fiber (14,700 c p s, -84 db) to discharge in excess of its spontaneous rate, while those within the inhibitory area prevented spontaneous discharge B Iso-intensity contour at -40 db level showing complete inhibition by tones between about 7,000 and 12,000 c p s

nerve fiber under observation. Figure 3A, in which the inhibitory area is limited to frequencies below the characteristic frequency, represents the findings in 2 of 6 cases studied in detail. In 2 others, the inhibitory area was located above the characteristic frequency, as shown in Fig 3B. In the remaining 2 cases, inhibitory areas both above and below were found.

An inhibitory area may encroach upon the anticipated response area of certain fibers. Response areas were usually determined by sweeping through

spontaneous activity is maximally depressed by the 9400 c p s tone, although some depression occurs for all frequencies between 4000 and 10,000 c p s. At higher intensity levels (-30 and -20 db), both 4000 and 9400 c p s now excite activity in excess of the spontaneous rate, while the intermediate

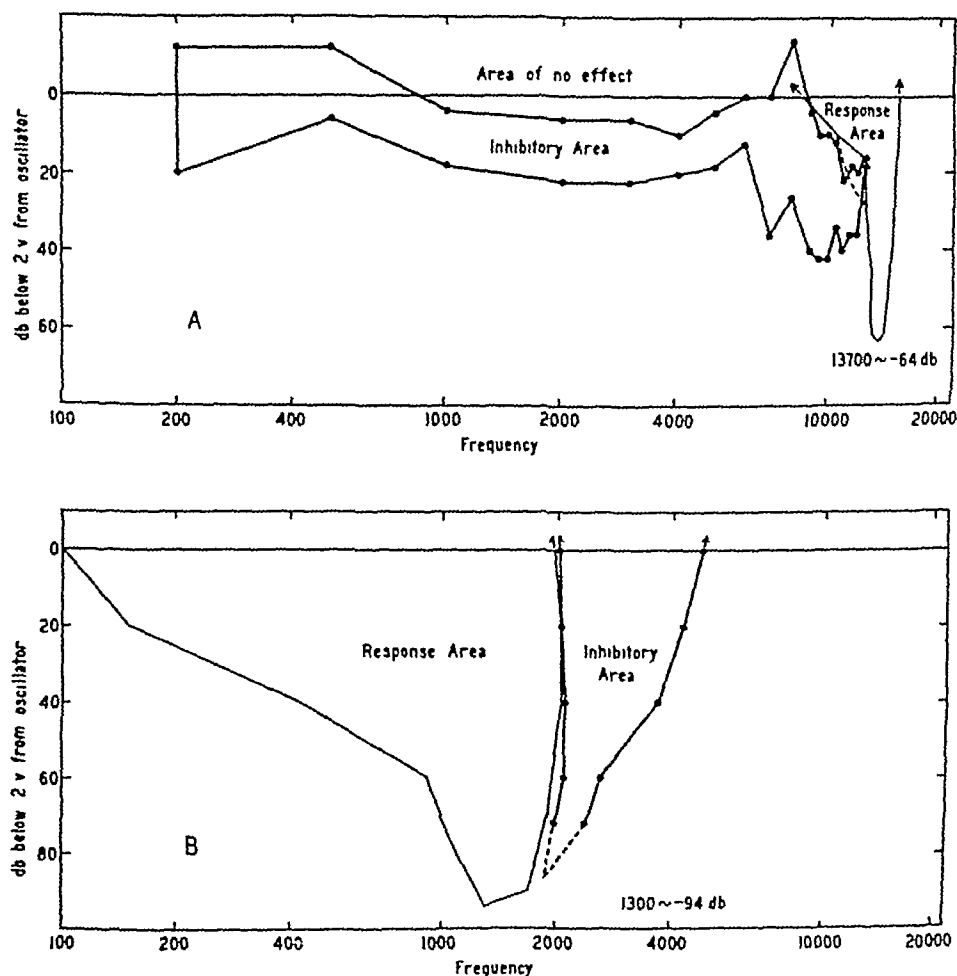


FIG 3 Inhibition of spontaneous discharge by acoustic stimulation. Comparison of tones which stop spontaneous activity (inhibitory area) with tones which increase activity (response area). Fiber A shows an inhibitory area (heavy lines) in the frequency range below the response area (light lines). Fiber B, from another cat, shows an inhibitory area in the frequency range above the response area.

frequencies continue to depress activity as before. When the tones are at 0 db, all frequencies excite.

In the above case, weak tones of the characteristic frequency were particularly effective in slowing spontaneous activity. This is not always true. The fiber of Fig. 1 and 2 is one of only two thus far isolated in which the

ing others. Thus at the onset of T_2 the baseline perceptibly widens while the number of large spikes decreases. This is interpreted as follows. The microelectrode, recording selectively from one fiber, also records unselectively from other fibers lying in the vicinity; in this instance the fiber yielding the spikes was inhibited while adjacent fibers were excited by T_2 .

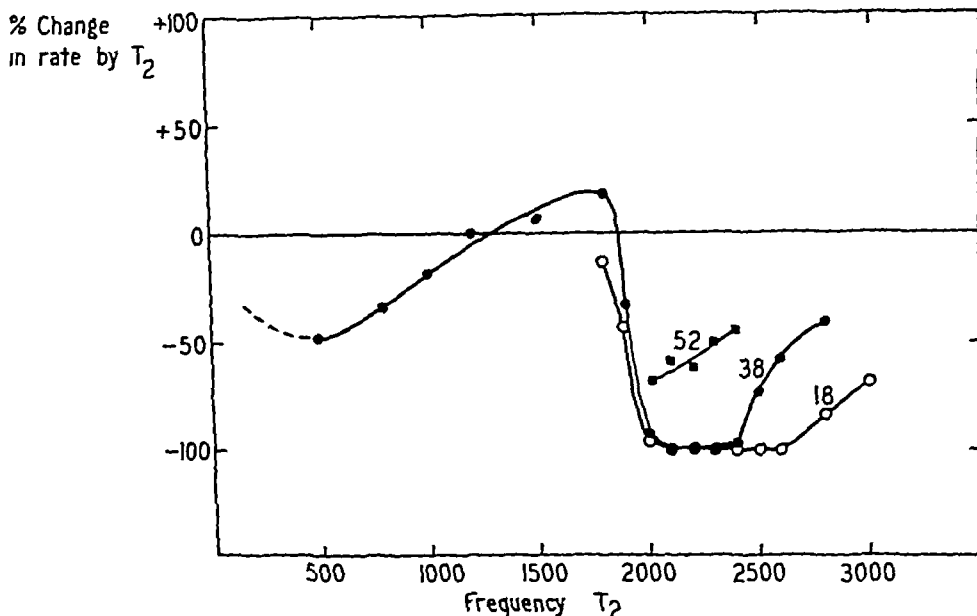


FIG 6 Inhibition of response to an adequate tone by a second sound. Iso-intensity contours for a fiber pictured in Fig 5a, showing effectiveness of T_2 (at frequencies given on abscissa and intensities given on contour lines) in inhibiting response to T_1 (1300 c.p.s., -72 db). Zero ordinate represents rate of discharge excited by T_1 (about 150 discharges per sec), and the points are plotted in terms of percentage change from that rate caused within the first second after onset of T_2 . Note sharp transition from excitation to inhibition by T_2 between 1800 and 2000 c.p.s.

The effectiveness of T_2 in inhibiting the response to T_1 depends upon the frequency and intensity of T_2 . This relationship is shown for one fiber in Fig 6 in terms of per cent change in the rate of the discharge excited by T_1 for different frequencies and intensities of T_2 . (Other data on this same fiber [1300 c.p.s., -94 db] are shown in Fig 3B and 5A.) At intensity levels of -52, -38, and -18 db there is partial or complete (100 per cent) inhibition of the response to T_1 by frequencies of T_2 between about 1800 and 3000 c.p.s. At the -38 db level a more complete range of frequencies of T_2 shows that in the region of the frequency of T_1 , the response is increased by T_2 , while it is decreased again by lower frequencies. At the -52 db level, T_2 does not completely obliterate the response to T_1 at any frequency. It is evident from this graph that a second tone may markedly influence the response of a fiber to the first tone. It may excite more discharges, or it may depress the

the frequencies at various intensity levels, and determining which frequencies elicited responses (see 5, p 43 ff) Usually the tones which excite can be enclosed in a roughly triangular area, but sometimes complex and irregular areas were observed. At first these irregularities were attributed to the inability of the sound system to maintain a constant level of intensity as the frequency was altered, but it now appears that this factor can at best only partially explain them. In Fig 4A, a typical irregular response area is shown

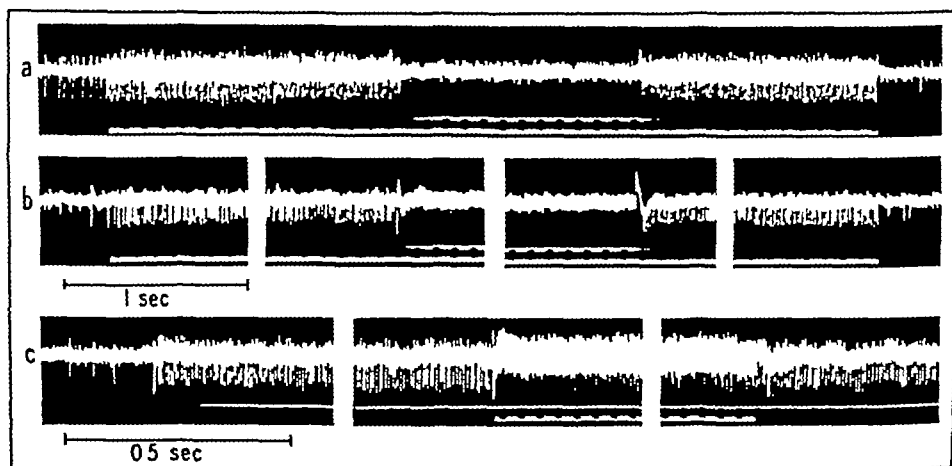


FIG 5 Inhibition of response to an adequate tone (T_1) by a second tone (T_2). Fiber a (1300 c.p.s., -94 db) and fiber b (1850 c.p.s., -88 db) are from the same cat, fiber c (10,000 c.p.s., -90 db) is from another animal. Duration of T_1 is indicated by the lower signal, and duration of T_2 by the upper signal beneath a and b. The reverse is true in c. The upper signal marks only approximately the onset and the end of the tone. For a, T_1 = 1300 c.p.s., -72 db, T_2 = 2300 c.p.s., -38 db. For b, T_1 = 1850 c.p.s., -60 db, T_2 = 2600 c.p.s., -26 db. For c, T_1 = 10,000 c.p.s., -78 db, T_2 = 7000 c.p.s., -60 db. About 1 sec. has been removed from the center of record b.

The tones which inhibited spontaneous activity are also shown as an inhibitory area. It seems clear that the response area deviates from a simple triangular shape because certain frequencies inhibit activity. Figure 4B, constructed from photographic records, shows quantitatively the extent of this inhibition at the -40 db intensity level.

2 a *Pure tones* *Inhibition of response to one tone by a second sound* The inhibitory effect of certain tones upon spontaneous discharge raises the question of whether the response of a nerve fiber excited by an adequate tone (T_1) can be modified by a second tone (T_2). This problem was attacked experimentally by intermittent stimulation with an exploring tone T_2 against a background of constant stimulation by a suitable T_1 .

Figure 5 illustrates complete or nearly complete inhibition by T_2 of the activity aroused by T_1 in three different fibers. Figure 5C is of particular interest because it demonstrates that T_2 may excite some fibers while depress-

depress activity, with the response area for a given fiber. The response area, it will be recalled, is made up of the single tones which excite a fiber. In Fig 7, frequencies both above and below the critical frequency are seen to be effective in inhibiting the response to T_1 . Furthermore, the inhibitory areas systematically encroach upon the response area, and for frequencies below the characteristic frequency, the response area may be abolished. In fact, *tones which excite when presented alone may actually stop the activity excited by other tones*.

Figure 8 shows the effectiveness of a wide range of tones in producing this inhibition. Each curve is for a different fiber, excited by an appropriate T_1 , and the degree of inhibition resulting from various T_2 s is indicated. For

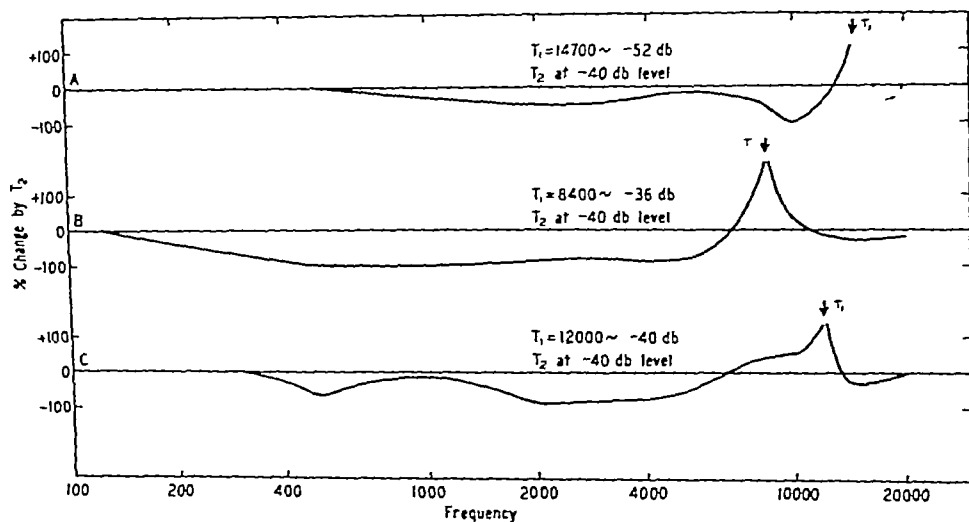


FIG 8 Inhibition of response to T_1 by T_2 . Three fibers (A = 14,700 c.p.s., -84 db, B = 8400 c.p.s., -78 db, C = 12,000 c.p.s., -66 db) from two cats. The graphs show how the response excited by a constant T_1 is modified by T_2 of various frequencies. Intensity level of T_2 is -40 db throughout.

example, in 8A, the discharge elicited by T_1 (160 spikes per second) was completely stopped by a T_2 of 10,000 c.p.s., -40 db, while a T_2 of 12,000 c.p.s., -40 db cut it down from 160 to 92 spikes per second (-43 per cent). The most striking feature of these curves is the remarkable effectiveness of tones of low frequency, tones throughout a range of 6 octaves below the characteristic frequency may inhibit (Fig 8B). In other words, if a nerve fiber is being excited by a high frequency, it can readily be stopped by low-frequency tones. This fact is apparent also in Fig 7A with its extensive low-tone inhibitory area.

It is pertinent here to compare for a given fiber the tones which inhibit spontaneous activity with those which stop discharge excited by adequate tones. Such a comparison can be made from Table 1, or from Fig 4A and 7A. For this fiber the T_2 inhibitory area both includes and extends beyond

response, and the effect observed is related to the frequency and intensity of the second tone

The duration of the inhibition produced by T_2 requires consideration. T_2 is always most effective immediately after it is turned on. Within a few seconds thereafter nerve discharges tend to reappear, and when the initial in-

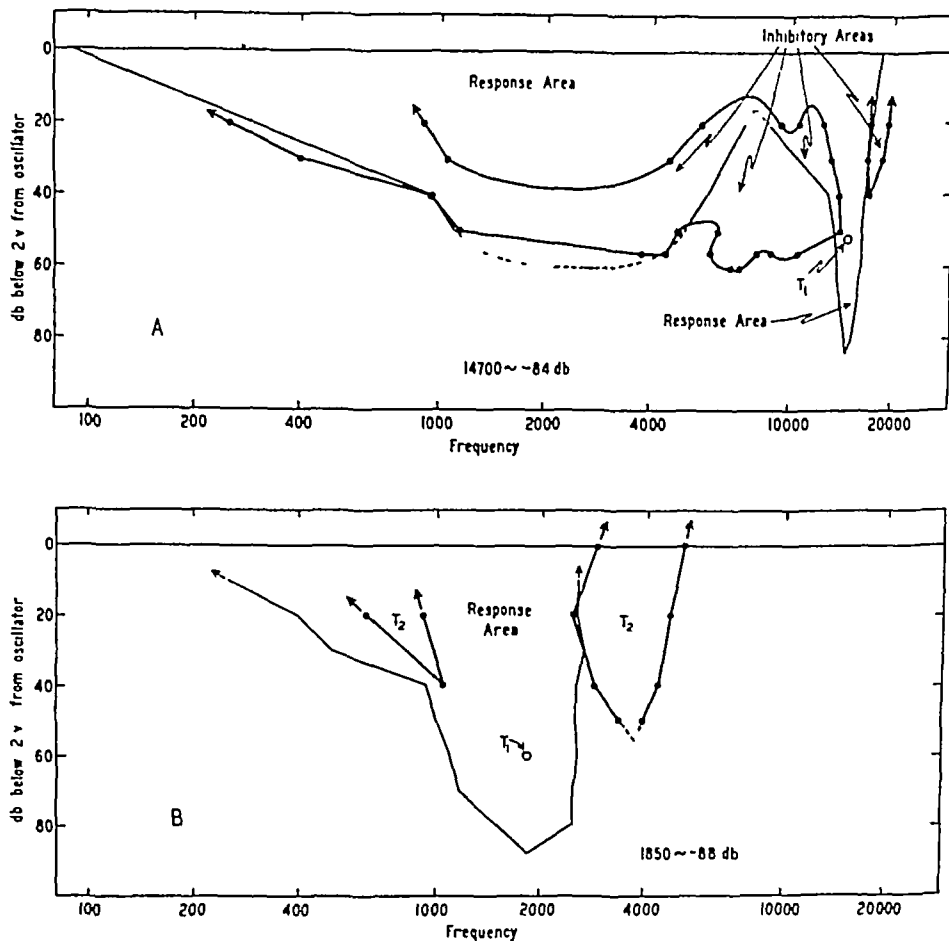


FIG 7 Comparison of inhibitory areas enclosing tones T_1 (inhibitory areas) with the response areas for 2 fibers from different cats. Frequency and intensity of T_1 as indicated. Maximum upper intensity limits of inhibitory area not explored. Note that the inhibitory area encroaches on the response area at low frequencies, and also at high frequencies.

hibition is only partial, the recovery may be almost complete. If T_2 completely inhibits the response to T_1 , however, there may be no nerve discharges whatever for as long as twenty seconds. Durations of T_2 longer than this have not been explored.

It is interesting to compare the inhibitory area, i.e., all of the T_2 's which

when corresponding inhibitory areas are determined. One such case, ated in columns 4 and 5 of Table 1, clearly demonstrates that the exnd, to some degree, the location of the inhibitory area depends upon₁ used. If T_1 is some octaves away from the characteristic frequency, inhibitory area may disappear completely. The quantitative data rel for the further elucidation of this important point are not available.

Noises. Some very suggestive inhibitory effects were obtained with a en rattle. This rattle is the type which produces a series of loud clacks as

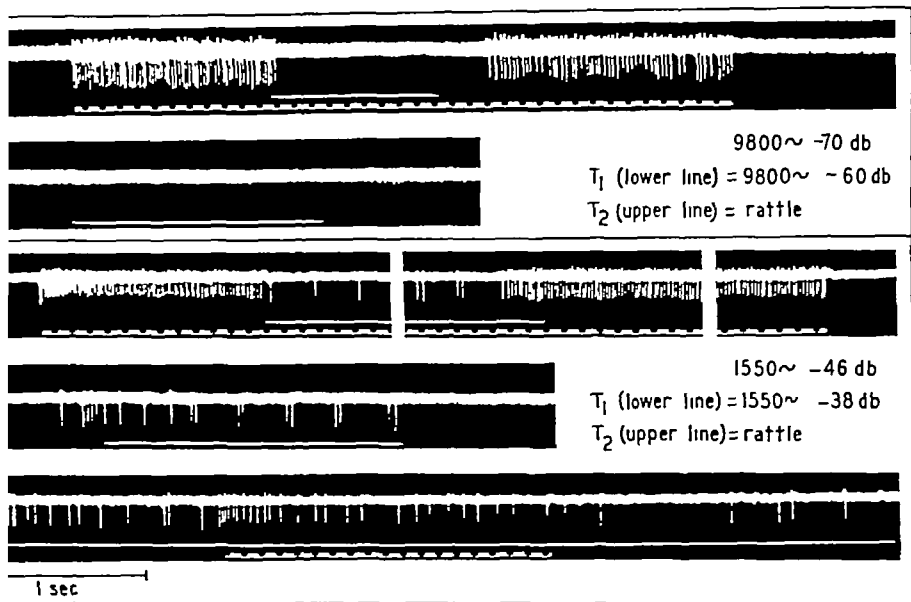


FIG 9 Inhibition of response to an adequate tone by noise (wooden rattle). There was spontaneous discharge in these fibers. a) the discharge excited by a tone of 9800 c p s, -70 db is abolished by the sound of the rattle. b) the rattle is sounded alone. c), d) illustrate the response of a fiber for which no pure tone (T_2) capable of inhibiting the discharge could be found. However, as shown in c, the rattle markedly reduced activity excited by a tone of 1550 c p s, -38 db. In d, the rattle alone is shown to cause a discharge. In e, the tone effective in c can only slightly modify the discharge excited by the rattle.

the tongue of wood snaps against a wooden gear when the device is twirled by hand. The rattle was sounded at a distance of about twenty feet from the animal and the noise entered the ear chiefly by way of the open bulla, since the external meatus was connected to the sound system and not open to the room.

The sound of the rattle effectively inhibited the response to pure tones of the fibers tested. Pure tones, on the other hand, are relatively ineffective in inhibiting responses to the noise of the rattle. Examples of these results, which are qualitative in nature because no frequency analysis or measure of intensity of the noise of the rattle was feasible, are shown in Fig 9. Record

the tones which inhibit spontaneous discharge. This appears to be the general rule. Fibers have been observed, however, in which no inhibition of spontaneous activity by tones or noises occurred, although marked inhibition by T_2 could be demonstrated.

Table 1 Data on a single auditory-nerve fiber (14,700 cps, -84 db). All frequencies given in kilocycles to conserve space. Columns 2 and 3 give response and inhibitory areas, respectively, when a single tone is used. Columns 4 and 5 give inhibitory tones (T_2) which perceptibly decrease the response to the T_1 indicated at the top of the column. Data in part plotted in Figs. 4A and 7A.

Intensity level (db)	Tones increasing spontaneous activity (response area)	Tones decreasing spontaneous activity (inhibitory area)	Tones (T_2) decreasing the activity caused by T_1	
			$T_1 = 14.7 \text{ kc p.s.} -52 \text{ db}$	$T_1 = 4.0 \text{ kc p.s.} -40 \text{ db}$
-90	none	none	none	none
-84	14.7	none	none	none
-80	14.5 to 15.2	none	none	none
-70	14.0 to 15.7	6.4 to 7.0, 9.0 to 9.4	none	none
-60	13.7 to 16.2	6.2 to 11.5	6.8 to 7.2	none
-56			3.8 to 4.4, 5.9 to 8.0, 8.8 to 10.5	
-50	1.1 to 5.0, 13.5 to 16.2	6.0 to 12.0	1.15 to 4.8, 6.2 to 14.1	none
-40	0.94 to 5.8, 13.0 to 16.5	6.6, 7.2 to 12.5	0.94 to 14.0, 17.0	6.6 to 7.0, 8.6 to 10.0
-30		7.6 to 8.5	0.4 to 1.05, 4.5 to 13.3, 17.0 to 19.0	6.2 to 11.0
-20	0.30 to 7.3, 8.6 to 17.3	8.2	0.25 to 0.9, 5.5 to 9.3, 10.5 to 12.5, 17.4 to 19.5	6.4 to 6.6, 7.1 to 9.0, 17.5 to 19.0
0	0.009 to 18.5			

Thus far we have related inhibitory values of T_2 to a single value of T_1 . The selection of T_1 is perfectly arbitrary, subject only to the one condition that it be located within the response area. For convenience, a T_1 of the characteristic frequency and about 20 db above the minimal intensity was usually chosen and the inhibitory values of T_2 determined for it. Selection of different values for T_1 for a given fiber leads to somewhat surprising re-

ment chloroform was dropped into the middle ear to paralyze the intra-aural muscles. Inhibition was demonstrable in the auditory fiber for about five minutes thereafter, at which time the nerve response disappeared entirely, presumably due to penetration of the cochlea by the drug. A few minutes thereafter the nerve response returned and could still be inhibited by a second tone. It is believed that this experiment demonstrates that the inhibitory effect occurs when the intra-aural muscles are not functioning.

A second type of mechanical interference is that between two patterns of movement of the basilar membrane. The pattern of movement of the basilar membrane caused by any reasonably intense tone is relatively extensive (4, 5)

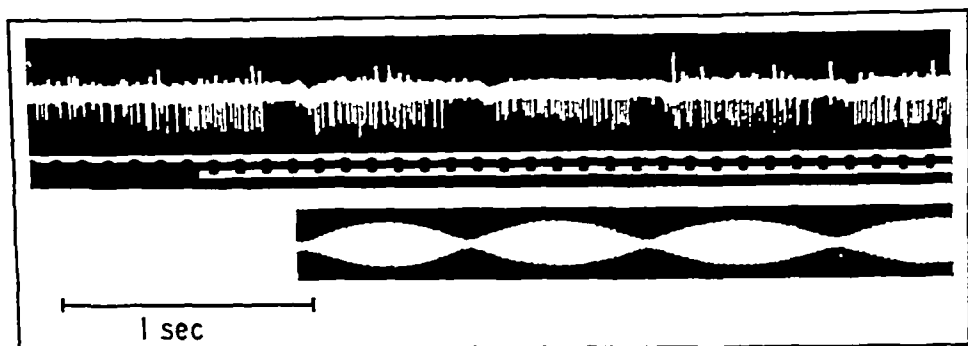


FIG 10 Response of an auditory nerve fiber to two beating tones T_1 (lower signal) = 1250 c.p.s., -60 db, T_2 (upper signal) = about 1250 c.p.s., -58 db. The upper record is from a 1250 c.p.s., -64 db fiber. The lower record shows how the beating tones appear to a microphone in the position of the cat's ear.

Two such extensive patterns must overlap throughout a considerable area, even when the exciting tones are widely separated in frequency. It might be suggested, therefore, that inhibitory areas for auditory fibers merely mark those regions of the auditory spectrum where two tones interfere with and effectively cancel one another on the basilar membrane.

Closer consideration reveals the inadequacy of this hypothesis. If we admit that movement of a particular portion of the basilar membrane is the adequate stimulus for a given auditory nerve fiber, then, to "inhibit" the discharge of the fiber in response to T_1 , it would be necessary for T_2 to cancel the movement *continually*. Alterations of the temporal pattern of movement that involve *momentary* periods of inactivity may occur, as when two tones of nearly the same frequency produce "beats," but the periods of inactivity alternate with other periods when the amplitude of movement is increased, and excitation should occur. Periodic interruption of the discharge of a nerve fiber by two beating tones can easily be demonstrated (Fig 10), but as the tones are separated in frequency the beats become more rapid and the discharge becomes more and more continuous.

Since inhibitory interaction between T_2 and T_1 is not confined to the

9a demonstrates complete inhibition of nerve activity by the noise which alone (record 9b) does not excite this particular fiber although it undoubtedly produces frequencies which lie within the response area for the fiber (9800 cps, -70 db) Records 9c, 9d, and 9e are from a fiber which could be inhibited only by noise Pure tones even at the highest available intensities were ineffective Yet the noise, which excited the fiber when presented alone (9d), markedly reduced the discharge elicited by an adequate pure tone (9c) When, as in 9e, the fiber is excited by the noise, the pure tone has very little effect upon the response

Records 9c, 9d, and 9e summarize, therefore, a situation in which a noise by itself excites activity in the auditory nerve, yet, when sounded along with a pure tone, the noise prevents activity The noise seems to allow the tone to arouse a certain number of discharges only, these may be few in number (9c, 9d, 9e) or none at all (9a, 9b) This fact seems to have an important bearing on the phenomenon of masking, and will be discussed later

DISCUSSION

1 *Proportion of fibers showing the inhibitory effect* Of eleven fibers intensively studied with one tone for inhibition of spontaneous activity, six showed inhibitory areas (Fig 3) and five definitely did not In every fiber that we have explored with two sounds we were able to demonstrate inhibition of the response to the first sound Usually the effective second sound could be either a pure tone or a noise (the rattle) In a few fibers the only sound that caused inhibition was the rattle Of fourteen fibers studied in detail with two sounds, eight were spontaneously active in silence It is not yet clear why some fibers should be inhibited by a limited range of the auditory spectrum (*i e*, why they show a specific inhibitory area), while others are inhibited non-specifically and only by noises Nevertheless, these inhibitory effects are relatively simple to demonstrate under the proper experimental conditions * It should be pointed out that while the number of fibers on which this report is based is relatively small, strictly comparable inhibitory effects have been noted but not carefully studied in at least 25 other fibers

2 *a Mechanical interference Nature of the inhibitory effect* The inhibitory effects under consideration might arise as a result of some sort of mechanical interference in the auditory system A possible place for such interference to occur is in the middle ear, where contraction of the intra-aural muscles is known to depress the aural microphonics for certain frequencies (11) The contractions thus might conceivably decrease nerve excitation by impeding conduction across the middle ear, but it is almost inconceivable that the relatively non-specific activation of the intra-aural muscles could account for the very abrupt transition from excitation to inhibition illustrated in Fig 6 Neither could it explain the existence of sharply defined, but different, inhibitory areas for different fibers in the same animal And in one experi-

* Dr Karl Lowy (personal communication) has been able to verify these observations

part must also be recognized (1) The cochlear potential, or perhaps some "generator potential" (2), might modify nerve response significantly. No specific experiments exclude these possibilities which, however, would be hard pressed to explain the sharp boundary between inhibitory and response areas, particularly at high intensity levels.

A third explanation for inhibitory effects in the cochlea can be given in terms of direct neural connections between different parts of the cochlea, and such connections seem to exist. Thus, Lorente de N6 (6) has described "spiral bundles" of nerve fibers in the cochlea which run within the ganglion of Corti and "often give collaterals . . . which enter several regions of the cochlea lying far apart from each other." Earlier, Cajal (3) confirmed an observation of Retzius according to which bundles of nerve fibers run spirally along the organ of Corti between the hair cells and the basilar membrane, these were termed "spiral packets" of nerve fibers to which no function was assigned. Poljak (8) has also described these "spiral fibers" which enter a "plexus" under the organ of Corti and eventually innervate a group of hair cells at some distance from their point of entry. Poljak feels that the spiral fibers serve only a limited number of hair cells, as they pass down the length of the cochlea, however, they are stated to "give rise to fine twigs which lie imbedded between the hair-cells and are in intimate contact with them" (8, pp 24-25). Tello (10) has elegantly pictured these spiral bundles (cf especially his Fig 18 and 20).

Any one of the various anatomical relationships just described supplies a basis for the explanation of inhibition in terms of a peripheral, cochlear mechanism. Little is known concerning the origin, course and distribution of the fibers which make up the intraganglionic spiral bundle, and the same can be said for the internal and external spiral plexi. If we assume, however, that some or all spiral fibers both originate and end in the region of the junction between hair cell and afferent neuron, and that they convey impulses from one region of the cochlea to another, a very attractive explanation for cochlear inhibitory effects can be formulated.

Movement of the basilar membrane would, under this theory, excite fibers of both the afferent (*external spiral* and *radial* types of Lorente de N6, *direct fibers* of Poljak) and the spiral type. The afferent fibers convey their impulses centrally in the usual manner. The spiral fibers, on the other hand, convey impulses to different regions of the cochlea itself. At each region where the spiral fiber collaterals terminate, a reduction in afferent nerve activity occurs. In other words we assume that the spiral fibers, conveying impulses from one to another region of the basilar membrane, depress excitability at a point distant from the region set into motion by the stimulus.

The assumptions just outlined will adequately explain all the inhibitory effects described in this paper. Spontaneous activity could be reduced or abolished and a second tone might depress activity already going on if spiral fibers were involved. The main difficulty with the hypothesis is the technical problem of subjecting it to a critical test. The nature of the sup-

situation in which there is an exact harmonic relation between the two tones it follows that the phase relations between T_1 and T_2 may be continually changing. The change in phase must cause a continual change in the pattern of vibration of each element of the basilar membrane that is affected by both tones, and *continuous* cancellation of movement at any one point will not occur.

Inhibition by noise such as the rattle cannot be explained by cancellation of movement, for the noise itself must produce an inconstant pattern of vibration of the basilar membrane and continuous cancellation of any steady pattern produced by a pure tone is impossible.

Finally, spontaneous activity of the nerve fiber is sometimes inhibited by a single tone. There seems to be little possibility of mechanical interference in this situation.

b *Nervous interaction* Other possible explanations for inhibition in the auditory nerve have a neural basis. Under this heading, therefore, mechanisms will be considered by which nerve excitation arising at one spot in the cochlea might depress activity in nerve fibers originating in a region some distance away.

The inhibition might be produced by reflex action. It is conceivable that afferent impulses aroused by sound stimulation reflexly activate efferent nerve fibers through synaptic connections in the central nervous system. According to Lorente de Nó (6), Cajal has described in the auditory nerve the efferent fibers required by this theory. These fibers, called "centrifugal fibers," terminate around the cell bodies of the ganglion of Corti. These efferent neurons might be distributed to specific cell bodies and by some unspecified mechanism prevent the passage of afferent impulses through them. Thus impulses arising in the cochlea would ascend only as far as the ganglion of Corti. It should be noted incidentally that Poljak considers the "centrifugal fibers" to be efferent fibers to the autonomic vasomotor neurons and thus not directly concerned with audition (8).

The obvious test of this theory is to section the auditory nerve central to the recording microelectrode and test for inhibition thereafter. Several attempts to accomplish this have failed, for the procedure invariably destroyed the blood supply to the cochlea, or dislocated the microelectrode. * One animal was completely strychninized on the theory that this might modify the usual picture if efferent fibers were involved in the inhibition. The drug caused no change.

Another possible explanation has an electrical basis. The action current generated by a conducting neuron is known to modify excitability and conduction in adjacent neurons (7). It should be noted, however, that the physiological electrical activity causes chiefly a minor increase in excitability and rate of conduction, an effect which appears wholly inadequate for explaining inhibition in the auditory nerve. The chance that electrotonic factors play a

* Dr. Karl Lowy has successfully demonstrated inhibition both after blocking the nerve with cocaine, and after section (personal communication).

(Fig 5) In this case the inhibitory areas may encroach upon and overlap the response area. Thus, by simultaneously presenting appropriate second tones which prevent rather than produce discharge in the auditory nerve, it is possible markedly to restrict the range of tones which can excite a given fiber (Fig 7)

The inhibitory action of low tones upon the activity aroused by high tones is much more widespread than the corresponding inhibitory action of high tones upon low tones. A tone as much as five octaves below a high-frequency excitatory tone may inhibit the discharge excited by the high tone (Fig 3 and 8)

The mechanism of the inhibition is unknown. Mechanical and electrical factors in the middle and inner ear appear to be excluded as an explanation, nor does it appear to have a reflex basis. It is suggested that nerve fibers underlying the organ of Corti connect widely distant parts of that structure, and that they serve to reduce the excitability of regions distant from the one set in motion by the inhibitory stimulus.

The function of the inhibition is obscure, but it seems probable that in the case of "masking" it plays an important role. The tones which inhibit single nerve-fiber activity in the cat have the same general distribution as those which, in the human subject, are known to be particularly effective as masking tones.

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posed influence exerted by the spiral fiber upon the hair-cell or the afferent fiber lies, of course, entirely in the realm of speculation

3 *Physiological significance* Whatever the means by which inhibition of auditory nerve responses is accomplished, the existence of the phenomenon makes it necessary to reconsider certain widely-accepted interpretations of auditory mechanisms. While it is not intended to pursue this matter in detail here, the physiological basis of one of these—masking in the ear—warrants a brief discussion.

"Masking" occurs when perception of a sound becomes difficult because a second sound is simultaneously impressed on the ear. The current view on the mechanism of masking may be illustrated by a quotation from Steinberg and Gardner (9, p. 14): "The nerve fibers which are activated by the masking sound are ineffective in contributing to the loudness of a tone heard in the presence of the masking sound." Masking occurs, according to this view, because the masking tone itself activates the fibers ordinarily excited by the masked tone. The masking tone "keeps the lines busy" as it were, and the masked tone loses its identity (loudness) because that identity lies in the exclusive "use" of a particular group of nerve fibers.

One important feature of this explanation is that, in those fibers shared between them, the two sounds together produce as great or greater discharge than is aroused by either alone. This supposition seems to be exactly contrary to the experimental findings summarized in Fig. 5 through 9, where fibers are shown to stop discharging when a second sound is presented. If it be conceded that these figures illustrate processes underlying masking, then masking occurs not because fibers are kept busy discharging, but because they are prevented from doing so. Figure 9 offers particularly strong evidence for this argument. The rattle used there is extremely effective both in masking sounds for the human ear, and in stopping activity in the cat auditory nerve.

It is impossible as yet to say how significant "inhibition" may be in the explanation of masking, and only future study can reveal how extensively inhibitory effects are involved in such normal functions as pitch and loudness perception, and the analysis of complex tones.

SUMMARY

Further studies by micro-electrodes of the nerve impulses in single auditory-nerve fibers in cats show that the spontaneous discharge which occurs in some fibers in silence can be stopped by certain tones or noises (Fig. 1). The tones which inhibit fall into one or more clearly-defined "inhibitory areas" for each fiber, analogous to the "response area" which comprises the tones which excite. For some fibers the inhibitory tones are higher in frequency than the excitatory tones, for others they are lower (Fig. 3). Some fibers have inhibitory areas both above and below, while still others fail to show inhibition by pure tones.

The discharge excited by an adequate tone or noise also can be reduced or abolished by the simultaneous presentation of a second tone or noise.

THE SUPERNORMAL PERIOD IN THE RECOVERY CYCLE OF MOTONEURONS*

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A CERTAIN interest attaches to the search for the existence of a period of supernormal excitability in the recovery cycle of motoneurons and other neurones in the central nervous system. The demonstration of supernormality in the soma of such neurones would in the first place remove the grounds that now exist for the qualitative distinction between the reactions of the neurone and its axon, and place the motoneurone in the general category of tissues that show a complete classical recovery cycle. Beyond this, however, and the implications of physiological behavior that are to be inferred from the existence of a supernormal recovery phase, there is a further, and perhaps more important, phenomenon associated with the presence of supernormality. In a variety of peripheral tissues, such as axons, the sympathetic ganglion, and the myoneural junction, it has been demonstrated that a single subliminal excitation may create a local disturbance much like that following a propagated discharge, in which a period of lowered threshold is included, presumably analogous to the supernormal period, and produced by the same physiological processes (22). During this period a second stimulus, itself normally subliminal, may be now of threshold intensity and set up a propagated disturbance. This phenomenon may be considered to be a type of "temporal" summation, in that a series of subliminal stimuli arriving seriatim at the same locus on the tissue to be excited, may by this mechanism ultimately evoke a response.

The simplicity of this scheme, and the unequivocal demonstration of its presence in a variety of peripheral structures has naturally led to the suggestion that this same phenomenon may account for "temporal" summation within the central nervous system (2, 4, 5, 6, 11). Failure to demonstrate supernormality in the central nervous system, and the clear exposition of a completely different basis for "temporal" summation in certain motoneurons (24), has led to a general view that temporal summation of this type plays no part in the activity of the central nervous system (9).

Against such a view must always stand the useful neurophysiological concept that explanations of the functions of the central nervous system may always be sought in the behavior of the simpler peripheral structures, and the belief that a simple and effective means of temporal summation would hardly be abandoned completely in favor of a more complicated system. In addition the search for supernormality has not extended beyond a certain

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the most striking resemblance to a bigeminy rhythm in the heart (Fig 1), where also two beats in quick succession are repeated after a cycle longer than the normal interval. As far as bigeminy in the heart is concerned, the most plausible explanation is probably that which attributes the phenomenon to a supernormal phase of recovery following the first beat which so lowers the threshold that the second or "coupled" beat arises spontaneously. (18) The presence of supernormality within the motoneurone could account equally well for "doubling" in the soleus rhythm. To test this hypothesis we have investigated the influence on "doubling" in the rhythms of soleus and triceps motoneurons of those factors known to modify in significant fashion the supernormal phase of peripheral tissues, namely, temperature, acid-base equilibrium, and veratrine. Presumably, if spontaneous "doubling" appears by virtue of a phase of supernormality appearing during recovery of the motoneurone, warming, acidity, and administration of veratrine should magnify the phenomenon, while decrease in temperature, or alkalinity, should diminish the frequency with which it can be observed.

METHOD

Twenty-five cats were employed, decerebrated under ether anesthesia by the trephine method at the mid-collular level. Records of single units were obtained from the muscle by means of a concentric needle-electrode of the Adrian type, suitable amplification and photographic recording on paper by means of a General Electric oscillograph. Rhythmic discharge was induced by elicitation of the shortening reaction. Due to difficulties occasionally encountered in recording single units in hyperexcitable animals when cold, with veratrine, or at the limits of acid or base shifts, the number of soleus units was at times reduced by appropriate ventral root section. This was most satisfactorily achieved by total section of the left 7th ventral lumbar root.

Cooling or warming of the animal was achieved by means of blankets containing finely divided solid carbon dioxide, or by electric lamps, respectively. Acid-base changes were effected by means of intravenous injection of $N/20$ $-N/40$ HCl or NaOH. Due to the buffering ability of the animal, significant changes toward the acid side were not readily produced until the injections of acid were combined with respiration of a mixture of 5 per cent CO_2 and 95 per cent O_2 . Veratrine hydrochloride was injected intravenously in dilute solution in total amounts of 0.3–1.0 mg.

RESULTS

A Temperature The influence of body temperature on the phenomenon of "doubling" was most marked. Earlier observations on the frequency of the phenomenon were confirmed (Fig 2) by experiments in which a short series of rhythmic discharges was evoked by gentle attempts to flex the joint, or in which a tendon jerk was elicited against a background of a persistent stretch reflex rhythm. Doubled beats were noticed both on initiation of the reflex and after the pause following the earlier premature discharges caused by the tendon tap. The lower the body temperature the more frequently would doubling appear, while at higher body temperature reflex discharge would be more likely to start without doubling (Fig. 3).

A more striking effect was the facilitation of the appearance of long runs of doubled rhythms by diminution in body temperature. Figure 4 shows a triceps unit which at $39.7^\circ C$ showed doubling to but a minor extent. At

rather restricted group of neurones of somewhat specialized function, and where in fact there exists evidence for the presence of supernormality following an antidromic impulse (24). A further search for supernormality was therefore made in motoneurones of rather less specialized function in which neurone discharge is known to develop as a rhythmic response to the general excitatory background, and is determined as much by the recovery cycle of the neurone as by the intensity of excitation. Presumably such a neurone, firing in a largely self-determined rhythm, might be more likely to show su-

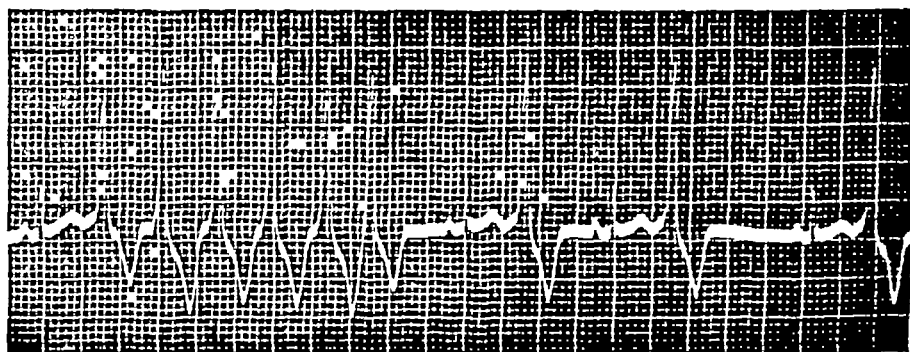


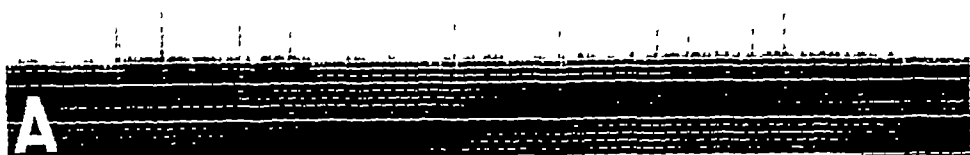
FIG 1 Cat Nembutal July 11, 1944 Bipolar electrodes attached at the base of the right ventricle and the threshold determined at a rectal temperature of 37.8°C , and an arterial pH of 7.2. There was no supernormal period, and no extrasystoles appeared unless the stimulus was turned on. Four cc. of 1/20 HCl were then infused intravenously, changing the arterial pH to 7.1. When stimulation was turned on, frequent ventricular extrasystoles appeared, and continued after the stimulation was discontinued arising in short bursts of tachycardia and coupled beats as shown here. Acid administration was slowed, the coupled beats disappeared, and a supernormal period was found in the part of the cycle at which the coupled beats occurred.

pernormal recovery, and the reflexes in which it is activated more likely to operate by means of temporal summation of the type found in peripheral tissue, than other neurones. The experiments to be reported here are concerned therefore with the existence of supernormality in the recovery cycle of triceps and soleus motoneurones operating rhythmically in the stretch reflex.

In soleus motoneurones firing rhythmically in the crossed-extensor reflex, Eccles and Hoff noted the occasional appearance of "doubled" discharges of the same unit (7). These occurred usually after the prolonged cycle following premature interruption of the rhythm of the motoneurone by an antidromic impulse, and have also been noted to occur at the first discharges as a unit is brought into rhythmic reflex discharge. From the nature of the action current and the effect on the rhythm of the neurone there was no question but that they represented a premature spontaneous discharge of the motoneurone rather than the chance discharge of another unit, or the polyphasic record of a single discharge recently described (3).

In their general effect on rhythmic pattern these doubled discharges bear

39.7 °C



35.5 °C.



— 0.1 sec

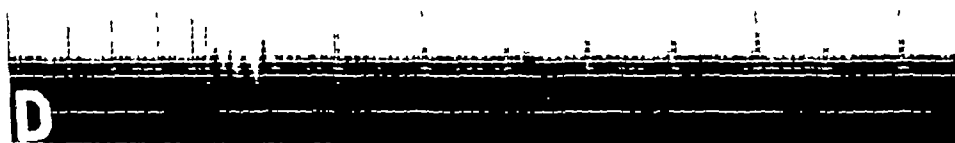


FIG 4 Sept 23, 1943 Decerebrate cat Triceps unit A, B Rectal temperature 39.7°C A Two reflex starts showing one double and one single start Twelve starts were recorded, 5 started singly and 7 doubled once only B Tendon taps against a stretch-reflex background No doubling in 8 recorded taps

C, D Rectal temperature 35.5°C The same unit showing persistent doubling at the start of a stretch reflex (C) and persistent doubling after an early tendon reflex discharge

35.5°C the unit doubled regularly on starting the stretch reflex, and on eliciting a tendon jerk, a previously single rhythm was converted into a persistent doubled rhythm

These doubled rhythms taken by themselves (see Fig 10) might appear to result from the chance association of two separate units, or from some unusual type of polyphasic record of a single unit (3), were it not for the

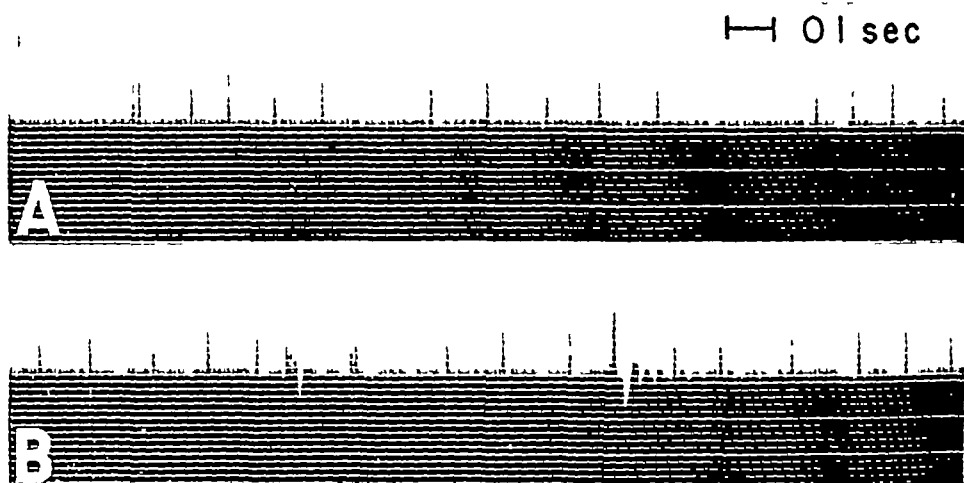


FIG 2 Sept 19, 1943 Decerebrate cat Rectal temperature 37.4°C Triceps unit A Three short bursts of rhythmic discharge elicited by gentle attempts to flex the elbow Of 16 reflexes recorded, 13 started with double, 3 with single discharge B Two tendon taps elicited against a stretch-reflex background Doubling after the earlier reflex interruption of the rhythm but not after a somewhat later premature discharge

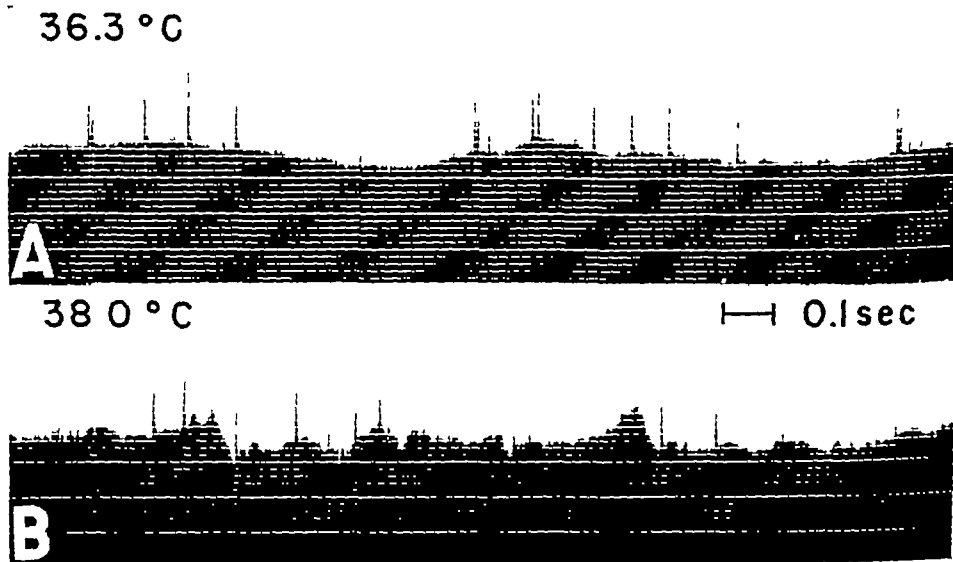


FIG 3 Oct 6, 1943 Decerebrate cat Left 7th lumbar ventral root sectioned Records from left soleus A Three reflex starts at a rectal temperature of 36.3°C , showing one or two doubles Of 28 recorded reflex starts, 25 doubled once, 1 twice and 2 showed no doubling B Two reflex starts at 38.0°C , showing no doubling Thirty-one reflexes were recorded at this temperature of which 28 were single and 3 doubled once only

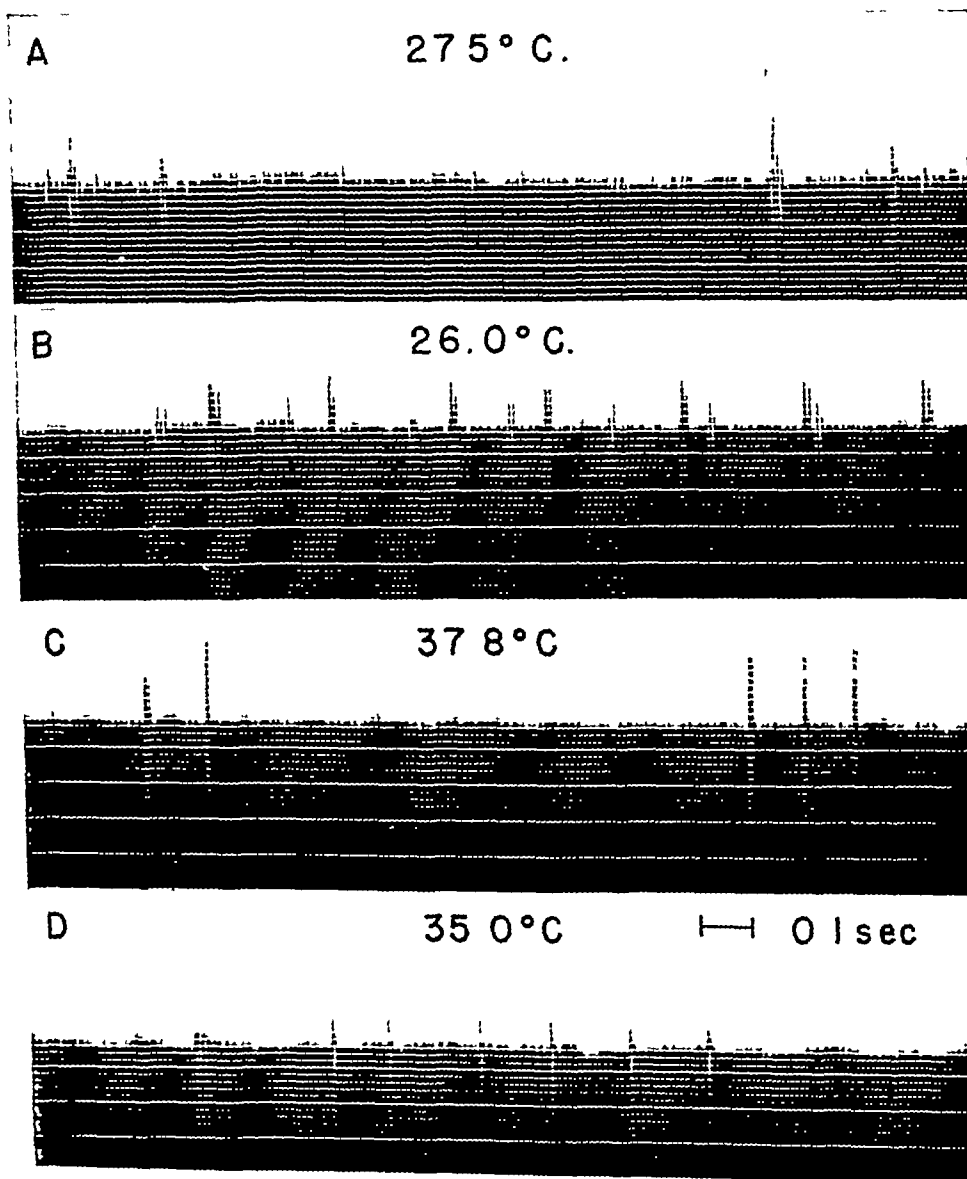


FIG 7 A Sept 17, 1943 triceps unit at 27.5°C B Sept 30, 1943 triceps unit at 26.0°C C Oct 8, 1943 triceps unit at 37.8°C All show double rhythms with triple starts In B two units are seen reacting similarly

D Oct 8, 1943 Soleus unit at 30.0°C, showing tripling at the onset of the reflex Timing indicated in D applies to all records

sistent doubled rhythms at a temperature (38.5°C) at which soleus units rarely show any doubling

This relative identity of reaction of the motoneurons within a single

— 0.1 sec

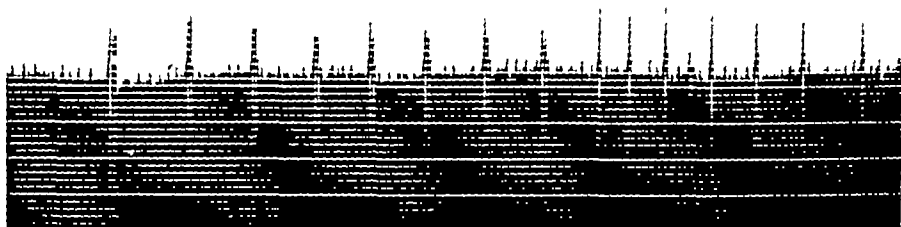


FIG 5 Sept 15, 1943 Decerebrate cat Triceps unit, temperature not recorded, showing change-over from double rhythm to single rhythm

fact that their relation to single discharges can clearly be demonstrated as in Fig 4D and in Fig 5 and 6

Of final interest in regard to the influence of temperature is the observation that as temperature fell, triple discharges were observed. Some instances of these are seen in Fig 7. Attempts to produce quadrupled discharges were largely defeated by the difficulty of retaining the isolation of single units in the spasmodic hyperexcitability shown by the decerebrate cat at lower temperatures. Figure 8 shows an example of a soleus motoneurone discharge at 34°C in which, despite the presence of other units, quadruple discharges may possibly be present.

In general, within a given motoneurone pool, various individual units reacted in much the same fashion. This may be seen in Fig 7B, where two triceps units may be distinguished, each starting with a triple response, and continuing with a double rhythm. Figure 9 affords a somewhat better illustration, inasmuch as it shows three soleus units starting out with fairly per-

— 0.1 sec

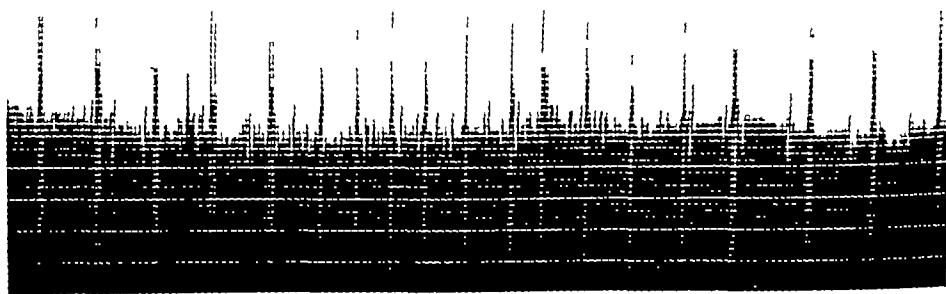


FIG 6 Sept 15, 1943 Decerebrate cat Soleus unit, unrecorded temperature, presumably between 35.0–36.0°C. This record shows the change from double discharge to single discharge as the reflex drive was increased by greater pull on the tendon, and the return to a double rhythm as the reflex drive was diminished.

Representative records in each experiment were measured by means of a dissecting microscope and ocular micrometer, to determine the interval separating double discharges. The least measured interval in a normal unit was 2.9 msec, in a triceps unit which continued to show doubling at 39.4°C. In the experiment with veratrine illustrated in Fig. 13 and 14, however, it was frequently found that an interval of no more than 2.5 msec separated in-

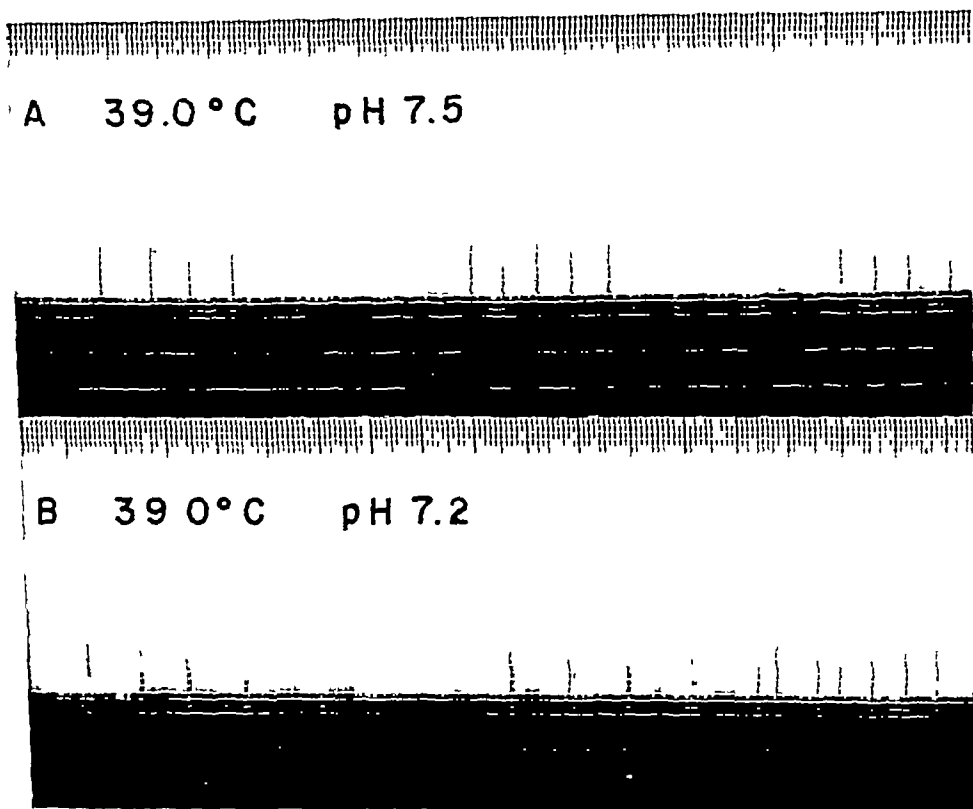


FIG. 12. July 4, 1944. Soleus unit at a rectal temperature of 39.0°C throughout the experiment. A. Control. Thirteen out of 13 reflexes started with single discharges. pH 7.5. B. After respiration of 5 per cent CO₂ and 95 per cent O₂ for 10 minutes during which 5 c c N 20 HCl was slowly infused into the right saphenous vein. pH 7.2. Twenty-six of 27 starts were doubled, and many short runs of double discharges were seen, two of which are shown.

dividual discharges, particularly when multiple firing occurred. In a great many experiments both soleus and triceps showed doubles at from 3.0 to 3.5 msec. The greater number of doubles ranged from 3.5 to 5.0 msec, however. Doubles at 6.0–10.0 msec were less frequent, though occasionally doubles were found at 12.0, 15.0, and 20.0 msec. Doubling after somewhat greater intervals may have been present in some experiments.

before triples could be recorded. Determinations of the pH of the arterial blood by means of a Beckman pH Meter, using the hypodermic type of sealed glass electrode, showed a drop from 7.5 to 7.2 in the experiment reported in Fig. 12.

C Veratrine The experiments with veratrine followed much the same pattern as those in which cooling and acid were employed. When units were

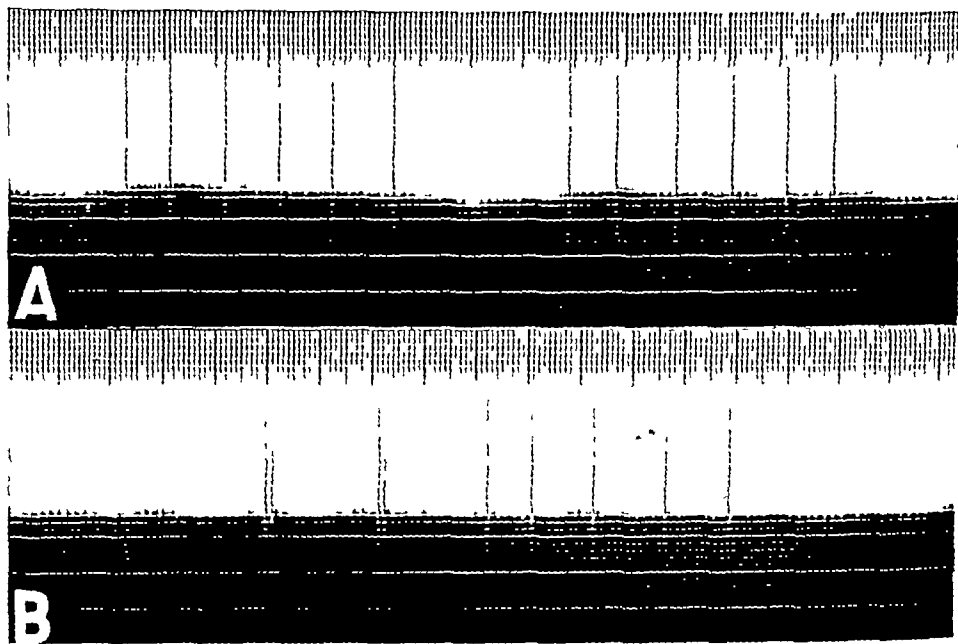


FIG. 11 June 2, 1944 Decerebrate cat. Left 7th lumbar ventral root sectioned. Rectal temperature 39.7°C throughout. A Control. Of some 100 reflex starts recorded or observed on the screen, there were no double responses. B After breathing 5 per cent CO_2 and 95 per cent O_2 for 25 min. with the slow intravenous injection of 15 c.c. N/20 HCl. All starts were doubles out of approximately 50 trials. On cessation of administration of acid and of breathing CO_2 , doubling disappeared, and reappeared where the acidifying procedure was reinstituted. Soleus unit.

isolated at temperatures at which doubling did not occur, it was found possible to evoke the phenomenon by the careful intravenous drip of veratrine acetate (0.05–0.1 mg. per c.c.) in total quantities of 0.3 to 1.0 mg. Because of the rapid appearance of general reflex hyperactivity, which seemed particularly marked in the extensor muscles, when more veratrine was given, well established double rhythms were not often seen (see, however, Fig. 13 B). The characteristic effect noted with veratrine was the appearance of multiple discharges. In a preparation in which a single pair of discharges alone was seen in control reflexes, three, four, five, and even six closely spaced discharges were observed (Fig. 13 and 14).

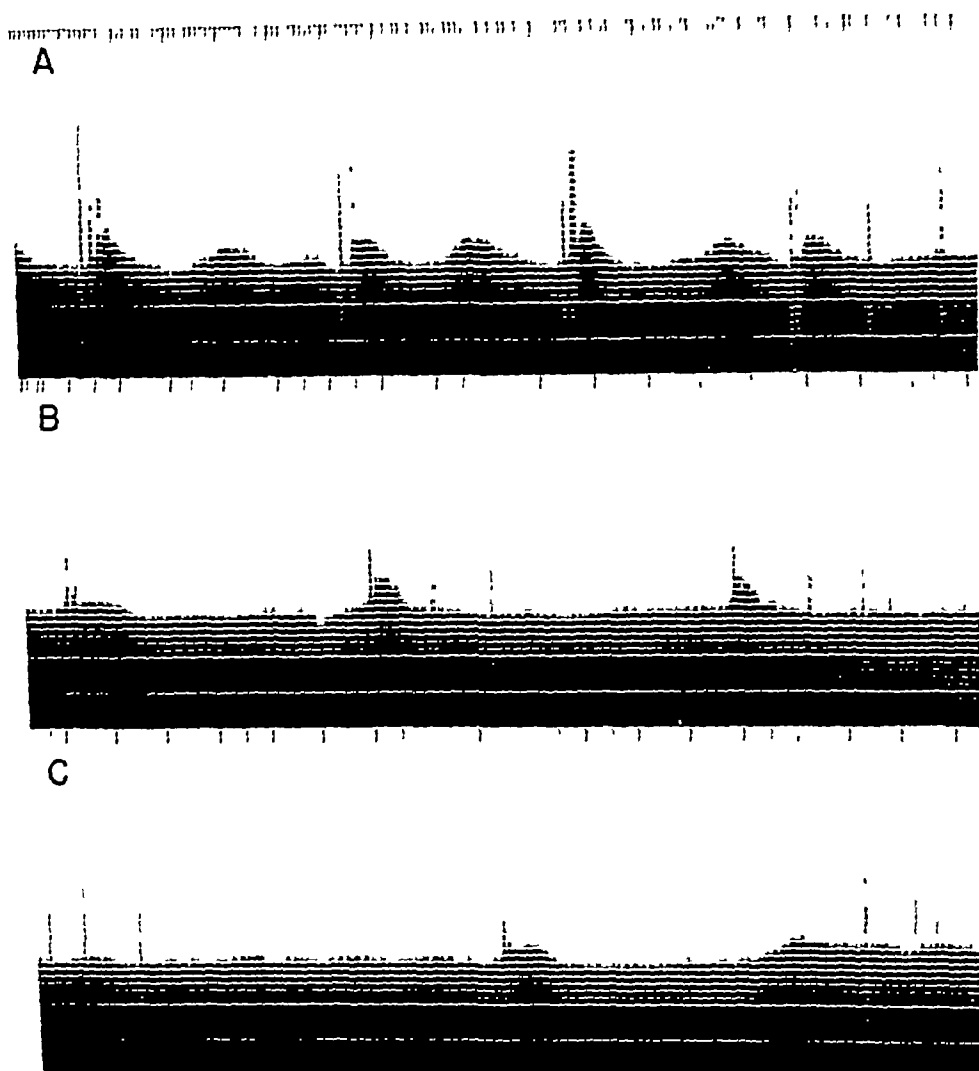


FIG 14 Continuation of Fig 13 In A are seen successively a series of six, four, six, and four discharges In B three reflexes consist of, or start with, five discharges, and in C a group of six closely spaced discharges is seen

FIG 13 July 8, 1944 Decerebrate cat Triceps unit Rectal temperature at the start of this experiment was 40.0°C , and rose slowly and progressively to 40.3°C by the end of the experiment

A Control A single double discharge was found at the start of 24 out of 27 reflexes recorded No triple discharges were seen in over 50 reflexes recorded or observed on the screen

B C-D Stages appearing as 0.4 mg veratrine acetate were slowly injected In B double rhythms appear, in C a pair of doubles are closely associated to form quadruples In D triple and quadruple discharges are seen

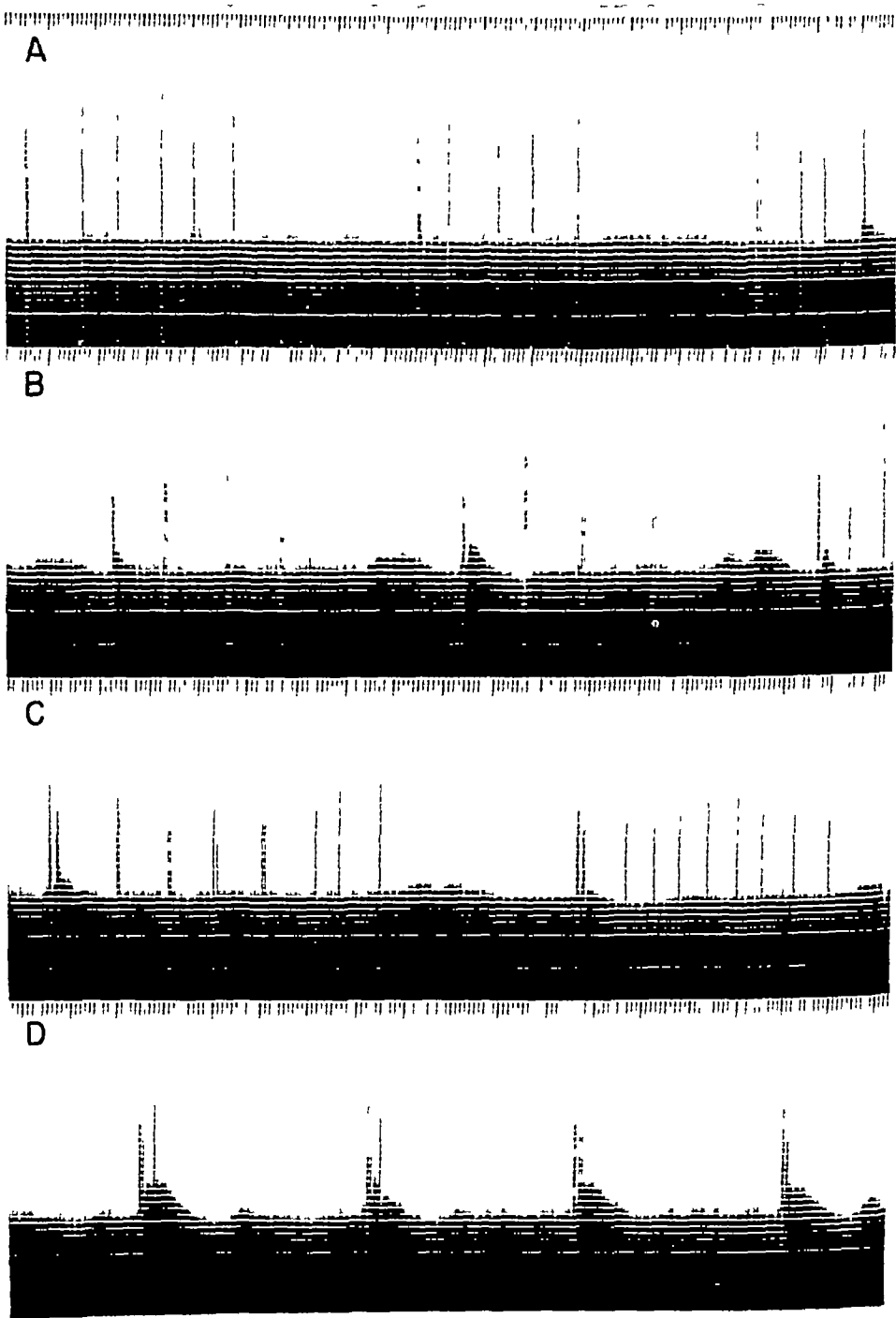


Fig 13 See opposite page for legend

The motoneurone will discharge a second time in quick succession, and cause the "doubling" we observe. The recovery cycle following this impulse will be modified significantly by the fact that during repetitive firing subnormality appears to summate, while supernormality does so to a lesser extent (8). Because of this, the supernormality of the previous cycle now becomes only a dip in the recovery curve, insufficient to bring the threshold below its normal level, and no further discharge occurs until, toward the end of the subnormal period, excitation level and threshold again meet. Thereafter, because of the moderate accumulation of subnormality, doubling disappears, until, following the premature discharge by a tendon tap, a long enough interval ensues for subnormality to subside, when doubling may again appear.

Factors increasing the intensity of supernormality may do two things. Doubling will persist longer and at higher levels of reflex drive because a greater accumulation of subnormality will be required to elevate the dip in the recovery curve above the normal threshold. The greater the reflex drive, and the more rapid the consequent rhythm, the greater will be the summation of subnormality, and the more difficult will it become to maintain a double rhythm. Thus a unit will double at the start, change to single discharge with more intense reflex drive, and will revert to doubling as the drive is lessened (see Fig. 6). When the supernormal period is even more intense, summation of subnormality resulting from two discharges will be insufficient to elevate the threshold sufficiently to prevent a third discharge during the supernormal period, and the theoretical limit to the number of quick discharges that might thus occur is not known.

The intervals which separate double discharges may serve to place time limits on the duration of supernormality in the motoneurone. The usual limits were from 3.0 to 15.0 msec, although occasional doubles at slightly shorter and somewhat greater intervals were observed. These are almost exactly the limits of supernormality observed in mammalian A fibers studied *in situ* by Gasser and Grundfest (13), Graham and Lorente de Nó (16), and Lorente de Nó and Graham (24). It indicates that, while supernormality may not always be present in motoneurons, when it is present it follows the same time course as it does in the axons derived from the motoneurons.

It is difficult to be certain about the biological usefulness of supernormality itself in these particular motoneurons. As has been pointed out by Gasser (8), supernormality fosters the development of rapid discharges in nervous tissues, as opposed to the slower rhythms regulated by the subnormal period. Triceps and soleus motoneurons are throughout the major part of their activity regulated by this latter mechanism in a relatively slow rhythm, approximately as fast as that of a rat heart beating at its maximum rate. The supernormal mechanism, however, makes possible a double or triple discharge at a rapid rate at the onset of the slower rhythmic discharge, or whenever the discharge has been halted momentarily. This in turn means

DISCUSSION

The experiments reported here do not constitute a demonstration of the existence of supernormality in the traditional manner of threshold determination. They present rather a series of phenomena that can best, and perhaps only, be explained by the presence of a supernormal period. Based on an analogy with bigeminus rhythms in the heart, they demonstrate that spontaneous double discharge of soleus and triceps motoneurons reacts in a predictable fashion to agents and factors known to modify significantly supernormality in peripheral tissues.

The only exception appears to be the influence of cold, which in isolated frog's nerve diminishes the duration and amplitude of the negative after-potential and presumably operates in the same manner on supernormality (10, 12). The association between the two may not always be complete (14, 15), however, and there may be considerable differences in the effect of cooling a nerve alone and cooling the entire animal. Moreover Katz (20) has shown that cooling frog's nerves facilitates the appearance of repetitive firing, which can be as well explained by the development of supernormality as by diminution in the rate of accommodation, although the distinction between the two processes may be hard to make. This problem is the same as that which must be faced in explaining the rhythmic oscillations noted by Lehman in nerve-fibers in alkaline or calcium-free media. Here rhythmic alternations between relative negativity and positivity occur and propagated discharges are set up at the negative crests. Such negative crests appear to resemble negative after-potentials, yet are produced by factors which usually reduce them. Obviously the problem requires further elucidation, it is plain however that the apparently paradoxical behavior of soleus and triceps motoneurons is not unique, and has similarities to that of nerve fibers.

While indirect, the evidence appears strongly convincing that in the motoneurons studied recovery through supernormality is a regular occurrence within the normal range of body temperature and pH. By extrapolation, one is justified to assume that, although supernormality in the strict sense may not be found invariably, there nevertheless occurs, as Lorente de Nó and Graham (24) point out, an early dip in the recovery cycle, which, although it does not bring the excitability below the normal threshold, nevertheless is a sign of the presence of those physiological reactions responsible for the appearance of supernormality itself in more favorable circumstances.

A postural motoneurone discharging for the first time under the impetus of reflex drive in the shortening reaction or crossed-extensor reflex presumably passes through the classical sequence of excitability changes. If supernormality is present, the threshold will drop below its normal level, and in the presence of a constant bombardment of impulses, one or a number of synaptic impulses arriving simultaneously within a certain area of unknown limits will constitute a threshold stimulus, and act as "detonator" impulses.

quantitative differences are present which lie at the root of the specialized functions performed by each neurone. This individuality of the various categories of neurones must restrain the temptation to consider one neurone, or one motoneurone, as a "sample" neurone.

SUMMARY

1 An analogy between the phenomenon of spontaneous "doubling" in the rhythmic discharge of soleus and triceps motoneurons and bigeminy rhythm in the heart leads to the suggestion that a supernormal period exists in the recovery cycle of these neurones.

2 Procedures known to enhance supernormality in peripheral tissue, namely, acidity, and administration of veratrine, increase the frequency with which spontaneous doubling appears, make possible the establishment of "double" rhythms, and permit "tripling" and even perhaps "quadrupling." With veratrine as many as six discharges in rapid series have been counted. Cooling also increases doubling.

3 The various phenomena observed may be explained best by the assumption that the neurones studied recover from a propagated discharge via supernormality.

4 The immediate usefulness of supernormality may be to permit the rapid development of muscular tension at the start of reflex activity.

5 The possibility is opened for the existence in the central nervous system of a type of temporal summation based on threshold lowering following a subliminal stimulus (the second phase of summation).

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the most favorable summation of muscular contraction, and forces the muscle to its maximum contraction in a shorter time than would be required if discharges were single and well spaced out from the beginning

The enhancement of supernormality by cooling suggests that this property of recovering tissue may constitute the basis for the efferent impulses noted in dorsal roots by Toennies (25), and shown to be enhanced by cold by Barron and Matthews (1). Were the synaptic endings of the dorsal root fibers to pass through a phase of marked supernormality, they might well be restimulated by the activity of the cells they themselves excited. The observations of Grundfest (17) and Ozorio de Almeida (25) might also be explained on the same basis.

It may well be, however, that the major function of the processes responsible for supernormality is to provide a simple mechanism for temporal summation of the type outlined in the introduction, and proposed by Eccles and others. This problem remains for further elucidation, the present work can be said only to bring back into discussion the possibility of such a mechanism.

The literature of temporal summation is not altogether clear as to the various possibilities that may exist. Fulton mentions but two. Actually, as Lorente de Nó makes clear, there are three. The first is the only one that can be called "true" temporal summation. This is the event that occurs in a peripheral nerve when two stimuli, individually subliminal, are delivered to the same region of the nerve at an interval less than that of the duration of the actual stimulus itself. For electrical stimuli this is an extremely brief interval and the refractory period of a nerve termination in the central nervous system must be great enough to prevent this type of summation to result from successive impulses delivered over the same synaptic terminals.

The second type of temporal summation is that made possible by the existence of delay paths within the central nervous system, and Lorente de Nó has presented a great part of the weighty evidence that this type of summation does in fact occur.

It is the third type which is rendered possible by the background of supernormality. Here there is no actual summation of stimuli, which occurs in the two preceding schemata. Instead, a first subliminal stimulus creates a local electrotonically propagated disturbance at one stage of which the threshold is lowered sufficiently for a second or some subsequent stimulus to be effective (the second phase of summation of Lorente de Nó). If such a mechanism participates in the reflex behavior of the cord, or in the more complicated reactions of the brain stem and cortex, it need not displace other mechanisms or be equally important in all circumstances. It is seen that a significant difference exists in the behavior arising out of supernormality between triceps and soleus motoneurons. If such differences can occur between two neurons of so closely allied functions, it can be imagined that far greater variations occur in other categories of neurons. This leads to the conclusion that, while qualitative similarities exist between neurons, vital

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THE NOBEL PRIZE IN PHYSIOLOGY AND MEDICINE FOR 1944

THE ANNOUNCEMENT from Stockholm on October 26th that the Nobel Prize in Physiology and Medicine for 1944 has been awarded to Joseph Erlanger and Herbert S. Gasser, both members of the Advisory Board of the *Journal*, will bring deep gratification to all those who, during the past twenty-five years, have followed the rapid growth of neurophysiology. The award stands as a highly significant tribute to neurophysiology in the broader sense, and it gives merited international recognition to two of the most gifted and resourceful investigators of this century. All will join in expressing great satisfaction that the distinguished prizes which had lapsed since 1939, can once more be awarded.

Professor Erlanger had been known to an earlier generation of physiologists for his outstanding work on the heart, particularly his illuminating studies on heart-block and on the dynamics of the circulation. His papers on blood pressure determination are classic. Soon after the introduction of the string galvanometer, he began to use the electrocardiograph, there followed a series of fundamental papers by Erlanger and his students which contributed notably to our understanding, not only of the events of the cardiac cycle, but also of the general nature of electrical potentials in living tissue. Thus when Dr. Erlanger some twenty-five years ago turned his attention to action potentials in nerves, he already had a broad background in electrophysiology through his earlier work on cardiac potentials. For many years Dr. Erlanger has been known as a stimulating teacher, much beloved, and those who have followed his writings can appreciate his industry and resourcefulness, also his record of over forty years of sustained productivity in experimental physiology.

Herbert Gasser joined the faculty of the Washington University School of Medicine in 1916, first as an Instructor in Physiology, later he was appointed Professor of Pharmacology. When war came he and Professor Erlanger threw their energies into a collaborative study of traumatic shock at the same time carrying on important work on the blood substitutes. When the war ended in 1918, striking new developments in electronic physics had been made, and Gasser, one of the first physiologists to explore the field, devised a cathode ray oscillograph which made it possible to record for the first time without distortion the rapid action potentials of nerve. In the first paper based on amplification, published with the physicist, H. S. Newcomer, Gasser described the thermionic vacuum tube amplifier with which they had analyzed the action potentials in the uncut phrenic nerve of the cat. (1) Eighteen months later appeared the classical paper of Gasser and Erlanger entitled "A study of the action currents of nerve with the cathode ray oscillograph" (2) Here for the first time were set forth correct time relations of the action potential, or spike potential as it is now called, the

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A NOTE ON THE TWO COMPONENTS OF THE DORSAL ROOT POTENTIAL*

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(Received for publication July 26, 1944)

A PRELIMINARY report by Dun on the dorsal root potential states that strychnine, while it increases the size of the potential, appears at the same time to make its latency longer. Such a combination of effects, however, seemed rather improbable, and the present work was undertaken to examine the matter further. Figure 1A reproduces a typical experiment on the spinal cord of the toad in which the potential was led off from the 3rd dorsal root while the stimulus was applied on the ipsilateral 9th root about 10 mm. away. The record of the potential under these circumstances presents in its rising phase two distinct steps betraying the presence of two components. This result is definite enough without strychninization (Fig 1A). But strychnine makes the separation of the two components clearer by acting on them differentially. Figure 1B taken 5.5 min. after the introduction of strychnine shows the large increase of the second component with no appreciable alteration of the first. Figure 1C taken after another half hour, at a more advanced stage of poisoning, displays the difference in a still more striking form. The second component is now not only greatly increased but also exhibits rhythmical oscillations, while the first component remains small. The whole potential when recorded with low amplification gives a picture in which the first component may be readily overlooked, giving rise to the impression of a large potential with a long latency. This in fact was the impression obtained by Dun (1). The separation of the two components can be rendered more striking by a further procedure which completely abolishes the first component without appreciably affecting the second. This is to make a transverse cut of the dorsum of the spinal cord midway between the leading and stimulating roots, the depth of the cut being about a quarter of the cord. Figure 1D shows the result of this procedure. It is seen that the first part of the potential is completely abolished, the curve starting only after the long latency of about 40 msec. The maximum is also reached later but there is only a slight diminution in its size. Making the cut deeper delays the maximum further (Fig 1E). But even when the cord is cut about three quarters across, there may be little diminution in the size of the potential eventually attained (Fig 1F).

* The work was done in Peiping in the summer of 1941. Dr. Dun is now in the Department of Psychology, National Southwest Associated University, Kunming. Dr. Feng is at present in the Department of Physiology, National Shanghai Medical College, Chungking.

gave the first indication of the existence of nerve components of different characteristics. Thus were the seeds sown for the rich harvest that followed. In 1924 came the proof, by one of the most ingenious and decisive experiments in the history of physiology, of the compound nature of the action potential in nerve (3). The continuation of this work led to an exhaustive determination of the constants of nerve fibers and the classification of nerve fibers according to their properties, much of the detail of which was summarized and extended in 1939 (4, 5).

The direct collaboration with Professor Erlanger ceased in 1933, when Dr. Gasser was called to the Chair of Physiology at Cornell Medical College in New York City and later (1935) to the Directorship of the Rockefeller Institute for Medical Research, which position he continues to hold. A spiritual collaboration between Erlanger and Gasser, however, still goes on, for each one—despite their separation and despite recent exigencies of war—has continued to elucidate important functions of the nervous system. Dr. Erlanger has devoted attention more particularly to further analysis of the phenomena of excitation and action in peripheral nerve fibers, in the belief that the complicated phenomena of the central nervous system will find their fundamental explanation in the relatively simple analogues of nerve conduction. Dr. Gasser, likewise equipped with the necessary insight into the properties of nerve fibers, has been able to make a frontal attack on the formidable problem of conduction in the brain and spinal cord.

Each has contributed his share to founding the neurology of the future. Just as Cajal and Golgi, Nobel Laureates of 1906, provided a fundamental understanding of the finer structures of the nervous system, and Sherrington and Adrian, Nobel Prizemen of 1932, elucidated the patterns of activity in the nervous system, so Erlanger and Gasser have been unfolding the mechanisms of that activity.

The work of these men and of the many students inspired by them is recasting the science of neurology in a new and more adequate mold, wherein time relations stand on an equal footing with spatial concepts in elucidating mechanisms of reaction.

THE EDITORS

27 October 1944

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tions directly from the ascending collaterals of the 9th root in the dorsal columns, while the second is produced by impulses pursuing a more devious course among the internuncial neurone chains between the two roots. For, assuming as is usual that the action of strychnine lowers synaptic resistances, a striking effect of this drug is only to be expected where there are many synapses to be traversed. And given a sufficient lowering of synaptic resistances, it is not surprising that the stimulation of the 9th root can exert its undiminished influence on the 3rd root even after their direct fibre connections in the dorsal columns are severed. The second part of the dorsal root potential recorded evidently has its origin in the activity of the internuncial neurones. Whether the first also involves internuncial neurones or not, cannot be so definitely stated. But it is in accordance with the economy of hypothesis to suppose that even the first part is due to certain internuncial neurones in the immediate neighbourhood of the 3rd root terminations activated directly via the ascending collaterals of the 9th root.

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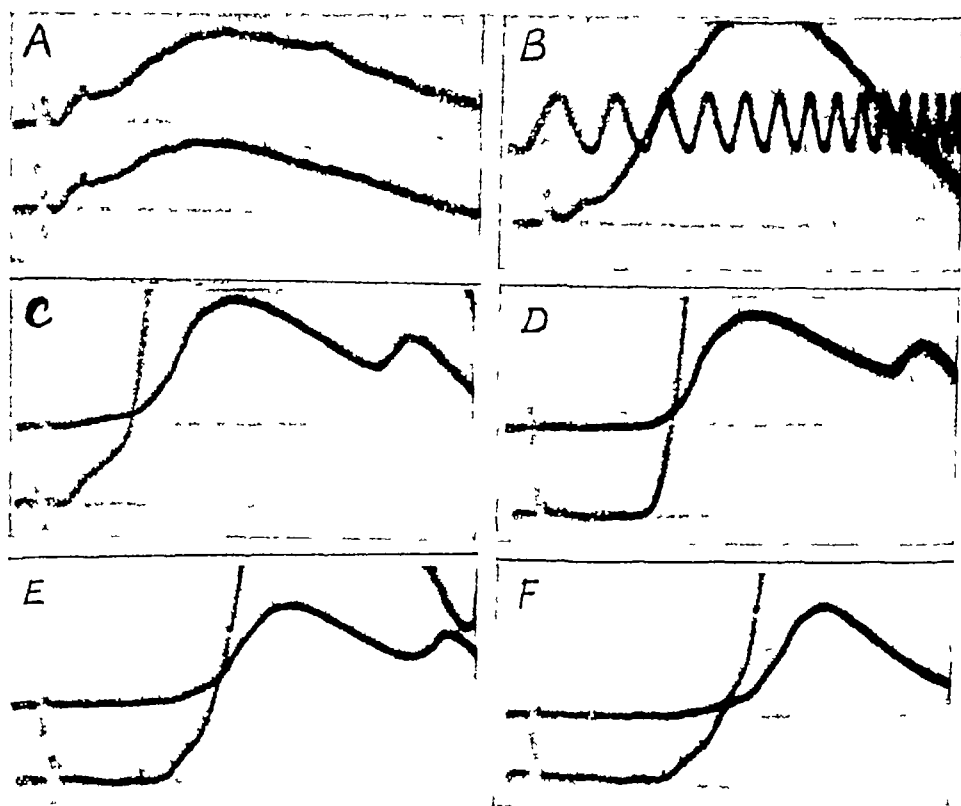


FIG 1 Cathode-ray oscillograph records of the dorsal root potentials in the toad, 22.5°C Stimulus on the 9th dorsal root, record from the 3rd dorsal root about 10 mm away. A, normal control taken at 10 45, 1 hr after dissection, 2 different strengths of stimulus. B, taken at 10 57, 5½ min after injecting 0.1 cc 1/300 strychnine intraperitoneally, time marks 50 cycles. C, lower curve taken at 11 27½, amplification same as in preceding records, upper curve 1 min later with greatly reduced amplification. These two amplifications are used in each of the subsequent records. D, dorsal ¼ of the cord sectioned at 11 39 about midway between 3rd and 9th roots, records taken 1–2 min afterwards. E, cut deepened to about ½ of the cord at 12 15, records ½–1 min later. F, cut further deepened to about ¾ of the cord at 12 18, records 5–6 min later.

DISCUSSION AND CONCLUSION

The potential in one dorsal root produced by the stimulation of another and distant dorsal root naturally divides itself into two parts. The first part has the characteristics of being not appreciably affected by strychnine but readily abolished by sectioning the dorsal columns of the cord between the stimulating and leading roots. The second has the opposite characteristic, *viz*, it is greatly enlarged and prolonged by strychnine but quite undiminished by the sectioning of the dorsal columns. To explain this difference, the most obvious supposition to make seems to be that the first part of the potential results from impulses reaching the region of the 3rd root termina-

RECEIVING AREAS OF THE TACTILE, AUDITORY, AND VISUAL SYSTEMS IN THE CEREBELLUM*†

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Medicine, Baltimore, Maryland*

(Received for publication July 31, 1944)

INTRODUCTION

IT IS AN established and universally recognized fact that the cerebellum is a place of convergence of impulses from proprioceptors located throughout the body. The great emphasis placed on this fact, the tacit assumption that the spinocerebellar tracts convey only impulses of proprioceptive origin, and the nature of signs and symptoms resulting from cerebellar deficit doubtless have combined to discourage serious experimental examination of the possibility that impulses from other groups of receptors also pass to this organ. Yet a number of considerations warrant the hypothesis that at least some classes of exteroceptors possess a cerebellar representation. The present investigation was begun when the following facts were considered together: (i) tactile impulses are relayed by the nuclei gracilis and cuneatus to the thalamus and thence to the cerebral cortex, and (ii) these same nuclei, according to many workers, send fibers to the cerebellum by way of the external arcuate fibers. Since there is no good reason for supposing that all impulses carried from the nuclei of the posterior columns by the external arcuate system originate only in proprioceptors, it seemed reasonable to attempt to determine whether impulses from tactile end-organs pass to the cerebellum.

The success which has attended recent attempts to map areas of sensory projection in the brain by recording evoked potential changes led us to use this method. It soon was found that the application of an appropriate tactile stimulus to the region of the foot of a cat evokes discrete potential changes in definite cerebellar areas. The extension of this original observation enabled us to secure evidence of the existence of a topical projection of certain parts of the cutaneous tactile system to the cerebellum.

Studies on the auditory system were initiated when it was observed that discrete surface positive potentials of latencies shorter than those of the tactile responses were evoked during displacement of hairs around the external ear. Since the mechanical stimulator used to move the hairs made a low clicking sound and since the evoked potentials proved not to be tactile

* Various aspects of this work were published in abstract form in 1942 (20, 21, 23, 24).

† The present paper was in manuscript form before the authors were aware of the publication by E. D. Adrian on "Afferent areas in the cerebellum connected with the limbs" (*Brain*, 1943, 66: 289-315). Although Professor Adrian evoked cerebellar responses by pressure, movements of joints, and stretching of muscle as well as by touch, the map which he has constructed of the afferent projection to the anterior lobe of the cat agrees in almost every detail with the one we obtained on the basis of uncomplicated tactile stimulation and presented at the meetings of the American Physiological Society in Boston in 1942 (21).

The stimulus used for studies on the auditory system consisted of a click from a loud speaker activated by a thyatron stimulator. The speaker usually was placed about twelve inches above the head and approximately equidistant from the two ears. Single or repetitive stimuli (1 per sec) were employed. In a number of cats, each under sodium pentobarbital anesthesia, the auditory area was explored after unilateral or bilateral destruction of (i) the auditory ossicles, (ii) the eighth cranial nerves, (iii) the fifth cranial nerves, or (iv) the inferior colliculi.

The stimulus used for studies on the visual system consisted of a low intensity flash from a 3 watt helium-filled neon lamp placed 1 to 2 inches from the pupil of one eye. The other eye was carefully shielded and did not receive any photic stimuli. The animal was kept in a dark room and atropine sulfate instilled in the eye. The light was arranged to flash once per second, since this interval was found to be longer than the recovery time of the system under study.

Delimitation of the cerebellar visual area was achieved in a series of experiments on cats under either chloralose alone or under a mixture of chloralose (60 mg per kg) and sodium pentobarbital (10 mg per kg). A surgical level of sodium pentobarbital anesthesia almost invariably suppressed completely the responses of this system in the cerebellum, and it should be emphasized that when a relatively weak photic stimulus is used the choice of an anesthetic is very important. In the course of this part of the work the visual area was explored before and after bilateral removal of the eyelids and extraocular muscles (3 animals). In addition, one chronically decorticate cat was used.

RESULTS

In reporting the results of a study devoted to mapping the cerebellar areas related to different peripheral receptors, it seems desirable to present them in a graphic manner. The figures which illustrate the results were constructed by placing photographs of the oscillograph traces on an outline drawing of the cerebellum at points corresponding to those at which they were recorded.

It should be emphasized that only the readily accessible portions of the cerebellar folia were explored. No attempt was made to place the pick-up electrode directly on any part of the extensive cortical surfaces which lie buried in the folial fissures. It is reasonable to suppose, however, that if such a laborious exploration were carried out it would reveal nothing topographically more significant than a series of inward extensions of each of the areas we have been able to map on the basis of responses which occur at accessible points.

1 *The tactile areas*

The electrical response evoked by a tactile stimulus and recorded from the pial surface of the cerebellum is one in which the predominant feature is a surface positive wave. Often the response is monophasic, and it is constant in appearance at any one point when evoked repetitively. Occasionally, the surface positive wave is preceded by a small surface negative wave. More frequently, however, the positive wave is followed by a negative excursion. Sometimes a triphasic response is observed in which the three components are first a negative wave, then a larger positive wave and, finally, a second negative excursion, the positive component is always the largest of the three when sodium pentobarbital anesthesia is used and this is usually the case when chloralose is the anesthetic.

in origin, it became evident that we were dealing with a representation of the auditory system in the cerebellum. Subsequent experiments adequately established this fact. Naturally the question then arose as to whether any other major exteroceptive system sends impulses to the cerebellum. Subsequent experiments were carried out to examine this point, and it was soon found that impulses of retinal origin reach certain cerebellar cortical areas.

METHODS

The present report is made on the basis of a study of approximately 150 cats. Studies on the monkey (*Macaca mulatta*) have been limited to the tactile system (22) and these observations only permit the statement that the responsive cerebellar areas in this species have essentially the same locations as those found in the cat. The cerebellum was exposed by rongeur-ing away the overlying bone and incising the dura. Any bleeding encountered in the course of the exposure was controlled by the usual techniques. In long experiments the exposed cerebellar surface was moistened with cerebrospinal fluid and the areas not under exploration were protected from drying by covering them with dura mater or with mineral oil. In each experiment all the accessible folial surfaces were explored. The most ventral structures, namely, lingula, flocculonodular lobe, and ventral parts of paraflocculi lobulus centralis, and uvula were not examined.

The evoked potentials were amplified by a three stage resistance-capacity coupled amplifier and were visualized on a cathode ray tube or recorded, when desired, by photographing the cathode ray trace. After trying various kinds of electrodes we adopted as the most satisfactory a unipolar one made of a saline-moistened cotton thread. The indifferent electrode was placed either on the muscles of the neck or in contact with the subcutaneous tissues of the neck, face or scalp. In all experiments the connections were such that electrode positivity was indicated by an upward deflection of the electron beam. The fast spontaneous electrical activity of the cerebellum was filtered out without distortion of the evoked changes.

The evoked potentials have been observed under several kinds of anesthesia. Although most of the successful experiments were done on cats under sodium pentobarbital (35 mg per kg) or chloralosan (70 mg per kg) or a mixture of the two, ether, chloralose, urethane, and chloral hydrate were tried singly and in certain combinations with sodium pentobarbital. All anesthetics except the ether, were administered by the intraperitoneal route. One chronic decerebrate cat under light sodium pentobarbital anesthesia was used and several acute decerebrate preparations were explored after withdrawal of the ether. In the study of the visual area use was made of a chronic preparation without neocortex. The rectal temperatures of the experimental animals were usually kept between 35° and 38°C.

Studies on the tactile system were carried out while stimuli were applied to hairs over chosen body surfaces by means of a small artist's brush rigidly attached to the moving armature of an electromagnetic device the coils of which were energized by a pulse of 3 to 5 msec duration. Arrangements were such that the movement of the stimulating brush occurred at a given point on the x-axis line of the cathode ray tube. The brush produced a quick displacement of a few hairs through a distance of about 0.5 mm. Thus the stimulus was the same as that used by Marshall, Woolsey, and Bard (14, 25) in their studies of somatic sensory mechanisms of the cerebral cortex. It is necessary to emphasize, as they have done, the desirability of using as a stimulus the abrupt displacement of a few hairs. In no case was the brush allowed to come into actual contact with the skin. The stimulus was repeated at regular intervals of 1 second, thus was found to be a rate sufficiently slow to ensure that each successive stimulus fell outside the recovery period of the system giving the evoked responses.

The tactile areas were mapped by means of two procedures. With the pick-up electrode at one position on the cerebellar cortex, responses were recorded as the tactile stimulator was applied seriatim to all parts of the body surface. In this way the relation of a given "point" on the cerebellar cortex to some portion of the tactile surface of the body was established. The first method was supplemented by exploring areas of the cerebellar cortex millimeter by millimeter with the pick-up electrode while the stimulator was fixed to displace a few hairs at a given peripheral locus. Thus, it was possible to determine which areas yielded maximal, intermediate, and minimal responses.

maximal responses usually lies between the sixth and seventh folia but we have been unable to determine with any certainty whether or not it continues throughout the depth of the fissure. The responses here show latencies

HAIRS AROUND FOOTPAD OF LEFT FOREPAW STIMULATED

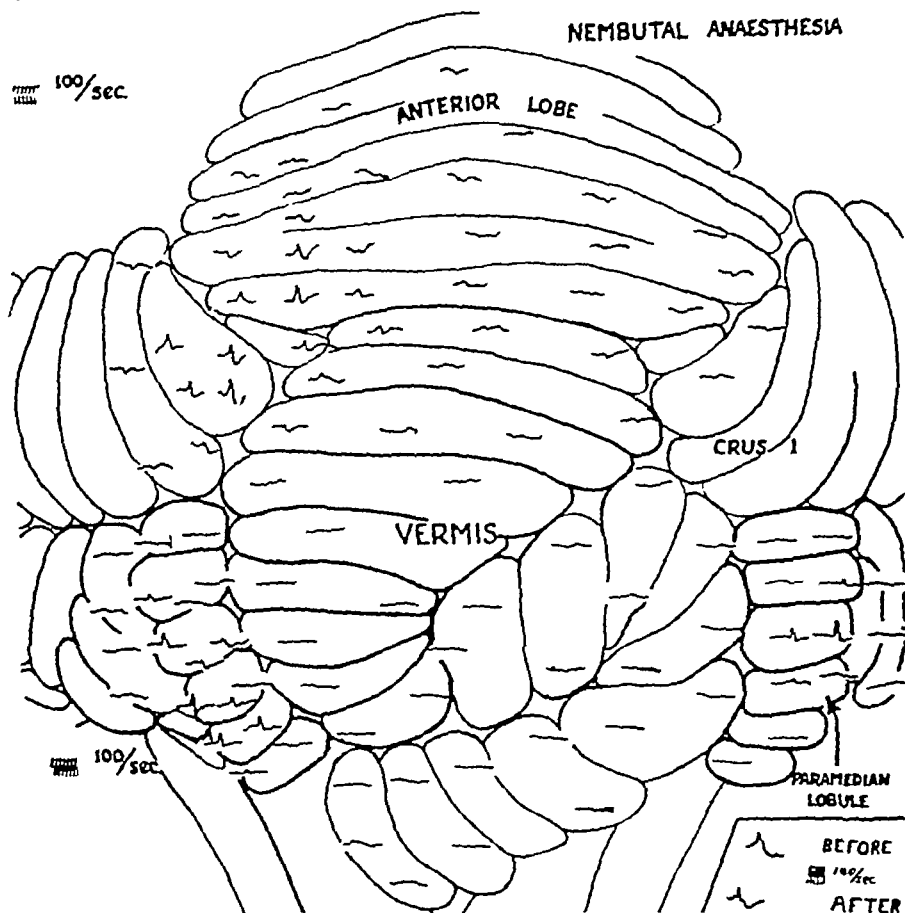


FIG 1. Distribution of potential changes evoked by brief displacement of hairs around pads of left forepaw of a cat. Sodium pentobarbital anaesthesia. There are three responsive areas: an anterior one centered at the posterolateral corner of the ipsilateral anterior lobe and extending into the adjacent parts of lobulus simplex and crus I, and two posterior areas, one in each paramedian lobule. In this and in all subsequent figures an upward deflection indicates a surface positive potential change. Upper time signal is for anterior responses, lower for those in paramedian lobules. Inset at lower right shows responses at an active point on anterior lobe before and after decerebration at an intercollicular level.

which range between 28 and 34 msec, in wave form they are identical to those of the forepaw area.

The responsive area in the paramedian lobule, which is related to the hindpaw, overlaps that of the forepaw area (Fig 4), but the region of maxi-

In order to eliminate the possibility of activation of pressure receptors or proprioceptors care was taken that the stimulating brush was in light contact with hairs and that it never actually pressed on the skin. Also, every precaution was taken to guard against movement of the animal's body as a result of either muscular activity or jarring of the animal board. Since the tactile stimulator operated with a faint click, it was essential to exclude the possibility that the source of the response was auditory. This was done by inclining the brush away from the hairs while leaving the head of the stimulator in its original position. If the response disappeared, it was regarded as of tactile origin, if it persisted, it was considered to be auditory.

That the recorded responses were not due to electrical spread along pial surfaces from underlying brain stem structures is evident from the fact that they do not persist as the exploring electrode is moved across cerebellar surfaces nearer and nearer to the brain stem. Their occurrence in decorticate and decerebrate animals rules out any essential dependence on structures cranial to the inferior colliculi.

Forepaw areas The loci of the responses obtained on slight displacement of hairs around the left forepaw are shown in Fig. 1 which presents the results of a typical experiment. There are three responsive areas: (i) the posterolateral part of the ipsilateral half of the anterior lobe, (ii) a portion of the ipsilateral paramedian lobule, and (iii) a smaller portion of the contralateral paramedian lobule. The first of these may or may not extend to include the most medial folium of crus I and the antero-lateral part of the lobulus simplex. The second occasionally extends into crus II.

The potential changes evoked in the tactile area of the anterior lobe are to a large extent surface positive, although, as can be seen, both surface negative and diphasic responses do occur. The latencies range from 14 to 17 msec. Responses recorded from the second tactile area (ipsilateral paramedian lobule) follow the stimulus by intervals of from 22 to 28 msec. Maximal responses were recorded most frequently along the lateral margin of the fourth folium, yet large surface positive and diphasic responses, initially surface positive, were also found at the lateral margins of the second to fifth folia. This area overlaps the hindpaw area of the paramedian lobule and occasionally extends into the most medial folia of crus II. The contralateral responsive area is slightly smaller and is located in approximately the same part of the paramedian lobule as the second area (Fig. 1). Here the latencies of the responses range from 42 to 46 msec.

Hindpaw areas Figure 2 illustrates typical tactile responses recorded from the pial surface of the cerebellum when hairs around the footpad of the left hindpaw are stimulated. Here, the responsive areas appear to be limited to the anterior lobe and the paramedian lobules.

The area in the anterior lobe extends over portions of the fifth, sixth, seventh, and eighth folia rostral to the primary fissure. This portion of the hindpaw representation is entirely ipsilateral, and it lies anterior to and wholly separate from the tactile representation of the forepaw. The area of

laterally, they average about 5 msec longer. The wave form is mainly surface positive and may or may not be preceded or followed by a small negative wave.

An area related to the vibrissae. An area of the cerebellum which appears to have a functional relation to the tactile receptors of the vibrissae is shown

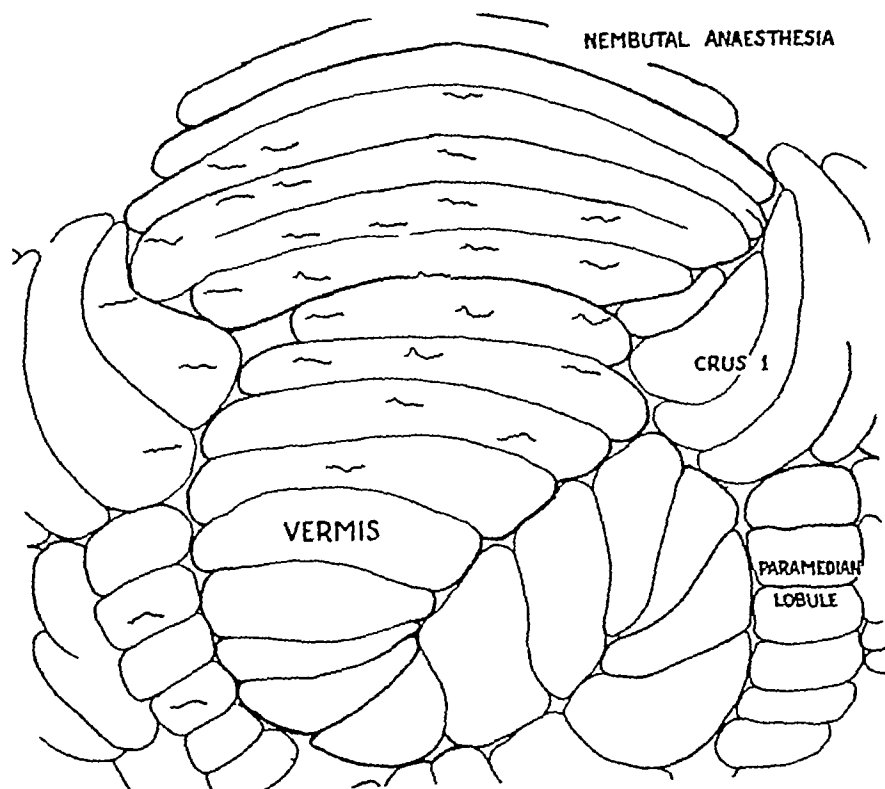


FIG 3 Distribution of potentials evoked by moving vibrissae of left side in a cat under sodium pentobarbital anesthesia. The area of activity extends on both sides of the primary fissure and is largely ipsilateral.

In Fig 3 The responses here are neither as pronounced nor as clearly localized as are those evoked by stimulation of the paws. This area lies near the midline and occupies four folia, two anterior to and two posterior to the primary fissure. The latencies of the responses vary from 10 to 14 msec. Usually the potential change is a purely surface positive one, but it may occasionally show a slow negative appendage. The responses seem to be predominantly ipsilateral in distribution, but the larger ones are found close to the midline and frequently a few appear contralaterally. They are abolished by section of the fifth nerve on the side of the stimulation (19). It is interesting to note that this area overlaps the anterior part of the auditory and visual areas which will be described in detail below.

mal responses usually extends one folium posterior to that of the forepaw and it is more apt to extend laterally into the most medial folia of crus II

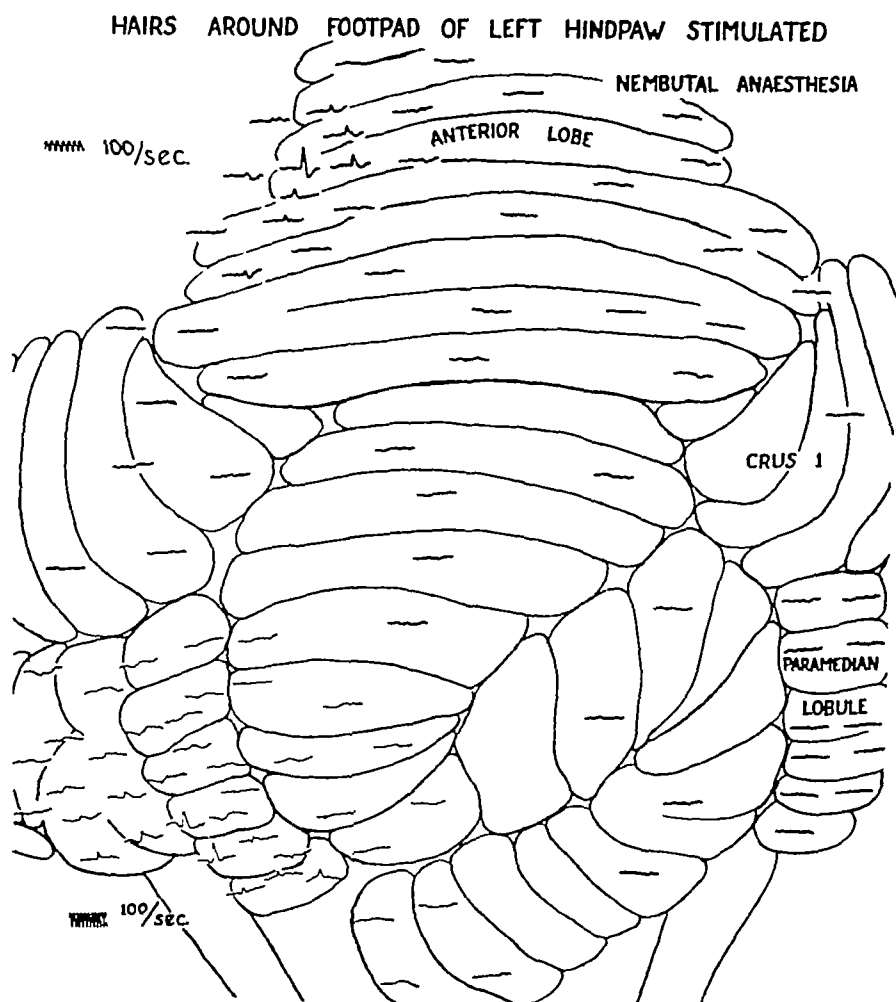


FIG 2 Cerebellar responses evoked by moving hairs around pads of left hind paw Sodium pentobarbital anesthesia Two areas of response are evident One situated ipsilaterally in the anterior lobe covers the lateral borders of the fifth, sixth, seventh and eighth folia rostral to the primary fissure A second area is located in the ipsilateral paramedian lobule and extends laterally into the two most medial folia of crus II A third area of very small responses may exist in the contralateral paramedian lobule Time signals upper for anterior lobe responses, lower for responses in paramedian lobule and crus II

It is unusual to find responses in the contralateral paramedian lobule but we have occasionally seen small waves in the third and fourth folia (Fig 2) The latencies of the ipsilateral responses range from 32 to 38 msec, contra-

in a marginal portion of an area the central part of which (where maximal responses would occur) is situated on the banks of fissures which we failed to explore

2 Auditory area

The results of a typical experiment on the cerebellar auditory area, carried out under chloralosan anesthesia, are shown in Fig 5A. All parts of the accessible cerebellar surface were explored. The principal feature of each of the recorded responses is a surface positive wave which is usually broken by two or more notches. The average latency of the responses is 11 msec, the range is from 6 to 14 msec. An intermediate region of minimal responses separates two larger zones where maximal responses occur. The larger of these is in the lobulus simplex and the anterior portions of the tuber vermis, the smaller lies posterior to it in the caudal part of the tuber vermis. The extent of the auditory area, as determined in a large number of cats anesthetized with chloralosan, is indicated in Fig 5B. It occupies midline cerebellar structures, *i.e.*, lobulus simplex and tuber vermis. Often, small responses are encountered bilaterally in the most medial two folia of crus I, in the anteromedial parts of the paramedian lobules, in the anterior portion of the pyramis, and in the posterior two folia of the culmen. But maximal responses (indicated by cross-hatching in Fig 5B) are restricted to the two zones mentioned above. Sometimes small surface negative waves can be recorded from the intermediate zone and from regions immediately adjacent to the periphery of the entire auditory area.

The depressing action of sodium pentobarbital on the auditory projection to the cerebellum can be appreciated by comparing the lower (C and D) and upper (A and B) halves of Fig 5. When an animal was studied under this anesthetic the total area of response was approximately one-half that when chloralosan was used, it is seen that the maximal potentials in the second turn of the tuber vermis and the small responses in the culmen, crus I, pyramis, and paramedian lobules drop out entirely. The responses which remain are principally surface positive waves, often monophasic, and they are never notched. Occasionally, a small negative component trails the main surface positive wave to form a diphasic potential change. Triphasic responses have never been observed when sodium pentobarbital was used. All responses obtained under this anesthetic are smaller in amplitude than those which can be evoked at the same loci when chloralosan is used. The latencies are the same with the two anesthetics. Despite the markedly depressing effect of sodium pentobarbital on the cerebellar responses it proved impossible to abolish them by increasing the depth of this anesthesia well beyond the level required for surgical anesthesia, in fact they did not disappear until the respiration had ceased. The part of the auditory area most resistant to the depressant action of this anesthetic is the posterior part of the lobulus simplex.

Other body areas In a large number of experiments careful attempts were made to determine whether tactile surfaces other than those of the paws and upper lip possess a cerebellar representation. It was found that electrical responses, smaller even than those recorded when the vibrissae were displaced, occur in the anterior lobe when discrete tactile stimuli are applied to

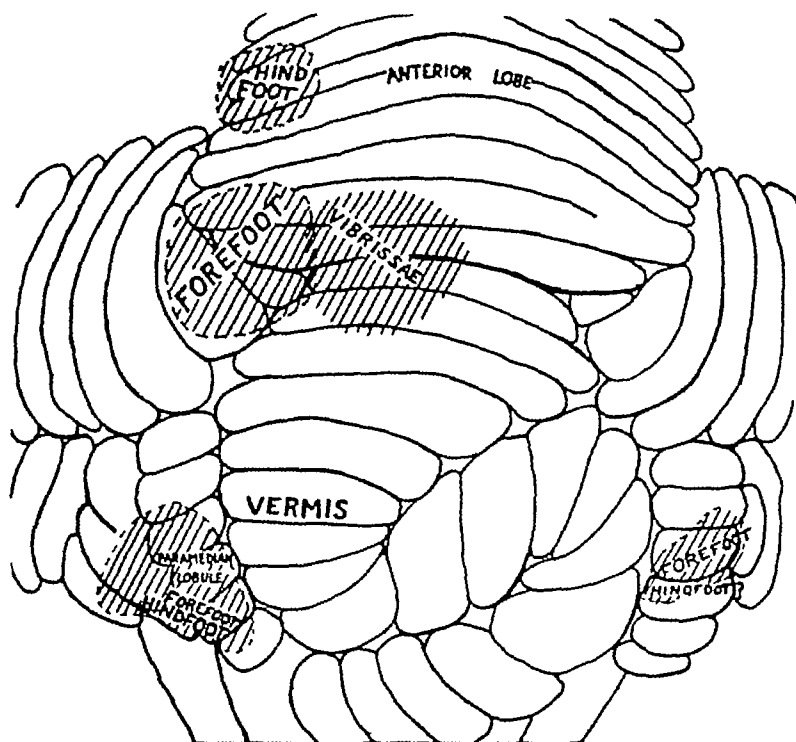


FIG. 4. Drawing of dorsal surface of cat's cerebellum showing tactile areas as determined by distribution of electrical responses evoked by stimulation of receptors in feet and upper lip of left side. Note overlap of forefoot and hindfoot areas of paramedian lobules and their complete topographical separation in anterior lobe. This drawing is derived from results obtained in entire series of experiments on tactile representation. See text for results of stimulation of parts of body surface not indicated in drawing.

shoulder, chest, back and hip. The arrangement appears to be one in which the representation of the tactile surfaces of one side of the trunk is situated medially in the ipsilateral half of the anterior lobe with hip at its forward extremity and shoulder overlapping the caudally placed head zone. The responsive area is somewhat more extensive, and the individual potential changes are a little larger, when the exploration is done under chloralosan or light ether anesthesia than when sodium pentobarbital is used. Again, one is faced with the possibility that the loci of these small electrical responses lie

When light sodium pentobarbital anesthesia is used the total responsive area is larger than when this anesthesia is deep, but it is somewhat smaller than that observed under chloralosan. Although the area of maximal responses is found on the lobulus simplex and anterior tuber-vermis just as when chloralosan alone is used, the second zone of maximal response in the posterior tuber vermis is practically non-existent, it appears that the ele-

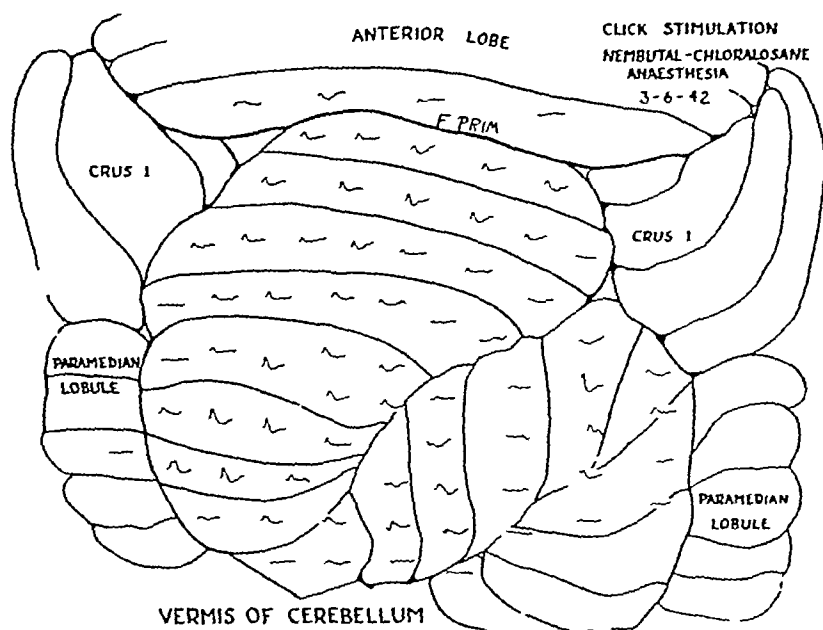


FIG 6 Cerebellar responses evoked by auditory (click) stimulation. Sodium pentobarbital (18 mg per kg)—chloralosan (35 mg per kg) anesthesia. Responsive areas are practically the same as when determined in cat anesthetized by chloralosan alone (Fig 5A), but individual responses are smaller and less complex

ments responsible for activity in this part of the auditory area are very susceptible to the depressant action of sodium pentobarbital

In determining the anterior limits of the auditory area, consideration must be given the fact that click stimuli evoke potential changes in the inferior colliculi. The occurrence of these responses in the tectum may lead to potential changes over the anterior lobe which are due to physical spread along the pial surface of the cerebellum. Indeed, as shown in Fig 7A, potential changes which are entirely due to this non-neuronal spread can be recorded from the greater part of the anterior lobe. They are surface positive, largest when recorded from an inferior colliculus, and they become progressively smaller as the exploring electrode is moved away from the tectum. In many preparations this spread onto the anterior lobe can be eliminated easily by breaking the pial bridge from colliculi to anterior lobe while in

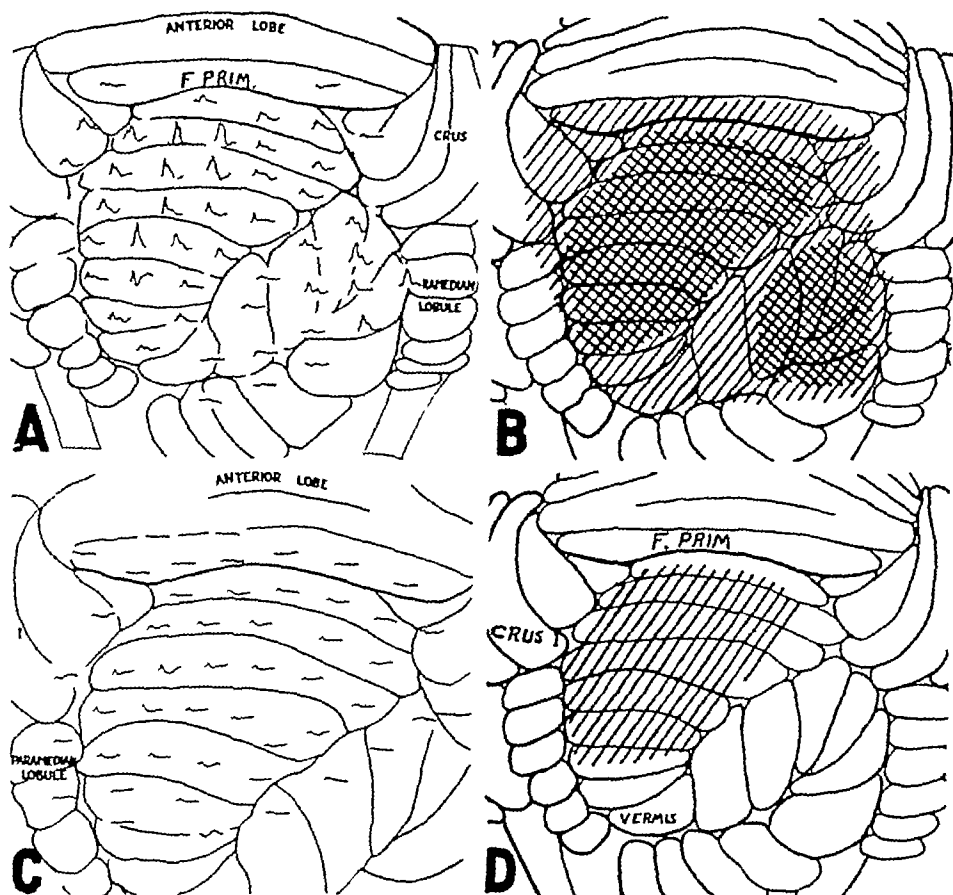


FIG 5 Drawings showing location and extent of auditory area of cerebellum of cat as determined under two anesthetics Click stimulation

A Distribution of responses in a cat under chloralose

B Composite chart showing extent of auditory area as determined in a series of cats under chloralose It occupies lobulus simplex and tuber vermis and extends into the most caudal folium of the anterior lobe and possibly bilaterally into crus I and into the right paramedian lobule The cross-hatched regions represent areas of maximal responses

C Distribution of auditory responses in a cat under sodium pentobarbital Same amplification as in A

D Composite chart showing extent of auditory area as determined in a series of cats anesthetized with sodium pentobarbital Compare with B

When full anesthesia is induced by giving a half dose each of sodium pentobarbital (18 mg per kg) and chloralose (35 mg per kg), the total responsive area is almost as large as when only chloralose is given (Fig 6) The two zones of maximal potential changes and a connecting region of minimal potentials are present, but the individual responses are smaller and less complex The latencies are the same as under full sodium pentobarbital or full chloralose anesthesia

it is seen that exclusion of impulses from one ear causes a retraction of the boundaries of the two major zones of response and a reduction in the size of the individual waves. Thus it appears that a definite summation effect is produced by activation of both cochleae. Also it is to be noted that after elimination of one cochlea the electrical responses evoked in the auditory area show much less notching. It may be suggested that at least part of the notching which is so conspicuous under chloralosan may be due to differences in latencies of the cerebellar cortical responses initiated by impulse

AUDITORY RESPONSES IN DECEREBRATE CAT

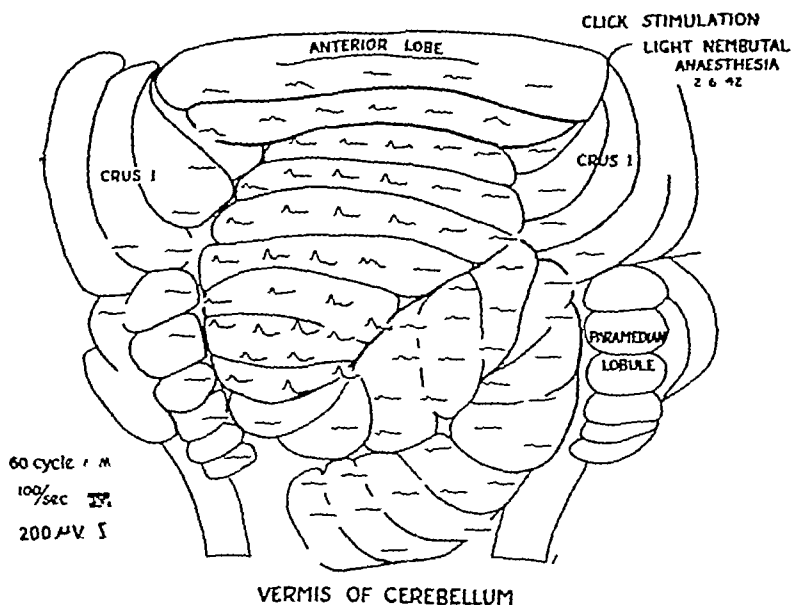


FIG 8 Distribution of auditory responses in cerebellum of a cat (No 114 of chronic series of Macht and Bard) which had survived decerebration for a period of 25 days. Trans-section passed from middle of superior colliculi dorsally to a level 1 mm rostral to pons ventrally. Click stimulation. Light sodium pentobarbital anesthesia. Amplifier gain same as in previous figures.

volleys from two ears which are not quite equidistant from the source of the sound.

That the responses of the auditory area are due neither to a physical spread of potential change from the temporal lobe nor to a cerebral relay in the pathways from cochleae to cerebellum was demonstrated in a number of experiments. The results of one of these are indicated in Fig 8. The chronically decerebrate cat used in this experiment was furnished by Drs. Macht and Bard, it had survived decerebration at a mid-mesencephalic level for 25 days and was studied by us under light sodium pentobarbital anesthesia. It can be seen that the cerebellar responses have the same characteristics

others it is necessary to insert a non-conducting barrier (*e g* , bakelite) which extends to but does not sever the superior cerebellar brachia. Needless to say, one or the other of these procedures was used routinely in all experiments involving the anterior portion of the auditory area.

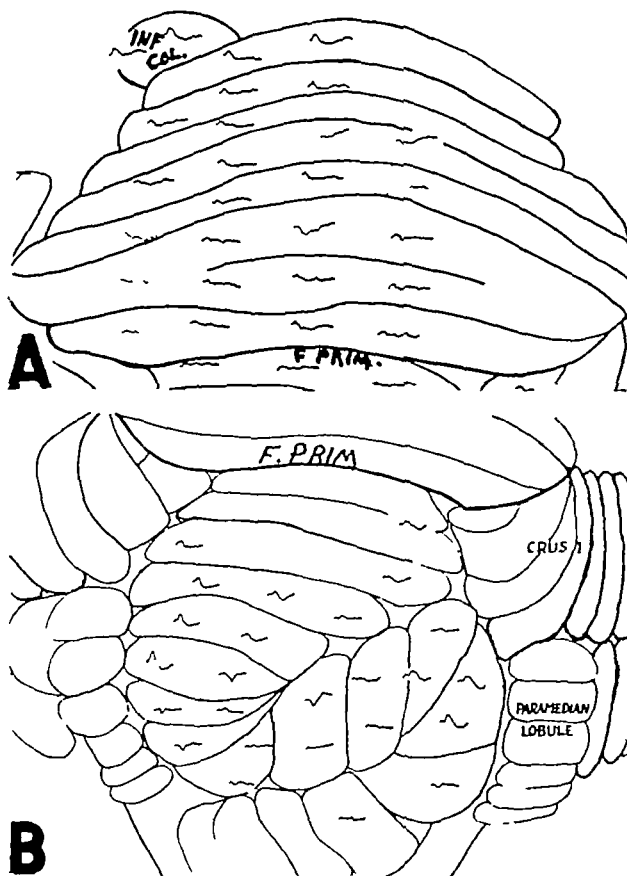


FIG 7 A Distribution in anterior lobe of potentials due to physical spread over pial surface from responses evoked in inferior colliculus by auditory stimulation. Note that potentials diminish in size as distance from colliculus increases. Sodium pentobarbital anesthesia.

B Responses to auditory (click) stimulation after destruction of middle and inner ears on the right side. Loud speaker 12 inches from left ear. Chloralosan anesthesia. Compare with Fig 5A. Note that while there are two zones of large potentials, responses are nearly absent from lobulus simplex and that wave form is less complex than when both ears are stimulated.

The extent and character of the auditory responses of the cerebellum when only one cochlea is stimulated by a click is shown in Fig 7B. In this experiment, carried out under chloralosan anesthesia, the middle and inner ears were destroyed on the right side. If Fig 7B be compared with Fig 5A

First, the auditory area was carefully mapped. Then the tympanic bullae were exposed by a ventral approach. Next, the responsive area was again determined and found to be exactly as it was before. Finally, the cochleae were carefully destroyed by means of a dental burr in such a way as to leave the semicircular canals intact, whereupon the cortical surface of the cerebellum was explored again. In no case did auditory stimulation produce any trace of a cerebellar response. That the cerebellum was still responsive was shown by the fact that typical potentials could be evoked in appropriate areas by tactile stimuli.

3 *Visual area*

In a large series of experiments it was found that stimulation of an eye by a flash of light evokes potential changes over a cerebellar cortical area which coincides very closely with the area which yields similar responses to auditory stimuli. Whether the representation of one retina differs from that of the other has not been determined, for we have not made a study in one and the same animal of the distribution of the cortical responses evoked from photic stimulation of each eye. A comparison of results obtained in a large number of experiments in which the left eye was stimulated with those secured in a somewhat smaller series in which the stimulus was applied to the right eye gives every indication that the two representations are nearly if not wholly continuous and have the same general pattern. In all probability differences in the details of the two representations exist, but a determination of the nature of these must be left to future work.

The visual responses, unlike the auditory, were only rarely obtained when sodium pentobarbital was the anesthetic. Consistent results were secured only when anesthesia was induced by chloralose (70 mg per kg) or by a mixture of chloralose (60 mg per kg) and sodium pentobarbital (10 mg per kg). From the very beginning of this work we were impressed and often disconcerted by the fact that the mechanisms underlying the cerebellar responses to photic stimulation are singularly subject to depression by most anesthetics. Their apparent immunity to chloralose may be the result of an excitatory or strychnine-like action of this anesthetic. Whatever the explanation may be, we are confident, for reasons which the description of our experiments will reveal, that the results obtained in cats under this anesthetic constitute valid evidence of the existence of a representation of the visual system in the cerebellum.

The area in which potential changes can be evoked by photic stimulation of an eye is indicated in Fig. 10, 11 and 12. It occupies chiefly portions of the vermis and exhibits variations which may conveniently be grouped in two classes on the basis of the number of zones of maximal responses. In most cats there are two, in some three such zones. One of these patterns is illustrated in Fig. 10B, the other in Fig. 11B. The maximal responses are rarely monophasic, usually diphasic, occasionally triphasic, but nearly always exhibit as their most prominent feature a surface positive wave. The most com-

and occur in the same places as in a normal preparation under light sodium pentobarbital anesthesia. Acute decerebrate cats proved to be not so responsive, probably because hemorrhage, "shock," etc., interfered with the afferent system under investigation.

The responses in Fig. 9 were obtained from a point on the lobulus simplex of a cat under chloralosan. They demonstrate the fact that as the intensity of the auditory stimulus is decreased, the magnitude of the potential change is decreased. It was also observed that when the intensity of the stimulus was held constant the size of the response decreased as the distance between the cat's head and the source of the sound was increased.

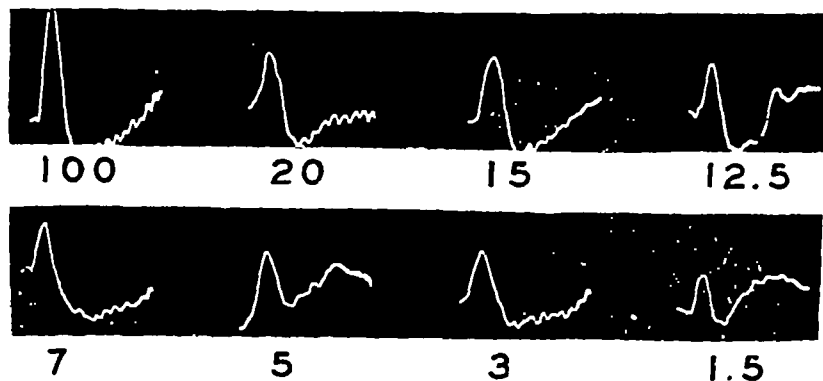


FIG. 9. A series of potentials recorded from the same spot on lobulus simplex. It shows the relation between the intensity of the auditory (click) stimulus and the size of the evoked response. The number under each trace indicates the intensity of the sound. The weakest stimulus (dial reading of 1.5) was a sound which could just be heard at a distance of one meter by an observer with normal hearing. The source of the click was a loud speaker placed 12 inches from the cat's head and equidistant from the two ears. Chloralosan anesthesia.

Bilateral destruction of the inferior colliculi abolished all responses evocable in the cerebellar cortex by click stimulation. Thus the pathways from cochleae to cerebellum must pass through or near these structures. This fact must be taken into consideration when acute decerebrate animals are used in studies of the cerebellar auditory area. If the level of transection is low or if the inferior colliculi are damaged negative results will be obtained.

Bilateral section of the fifth nerves did not change the size, characteristics or distribution of the responses. Bilateral section of the eighth nerves, of course, abolished all the responses. Bilateral destruction of the middle ear bones was found to reduce the amplitude of the responses, but it did not abolish them, presumably because bone conduction sufficed for adequate stimulation of the cochleae.

In order to eliminate further any possibility that factors other than auditory may be partially responsible for the evoked potentials, the following control experiment was done in a series of animals under various anesthetics.

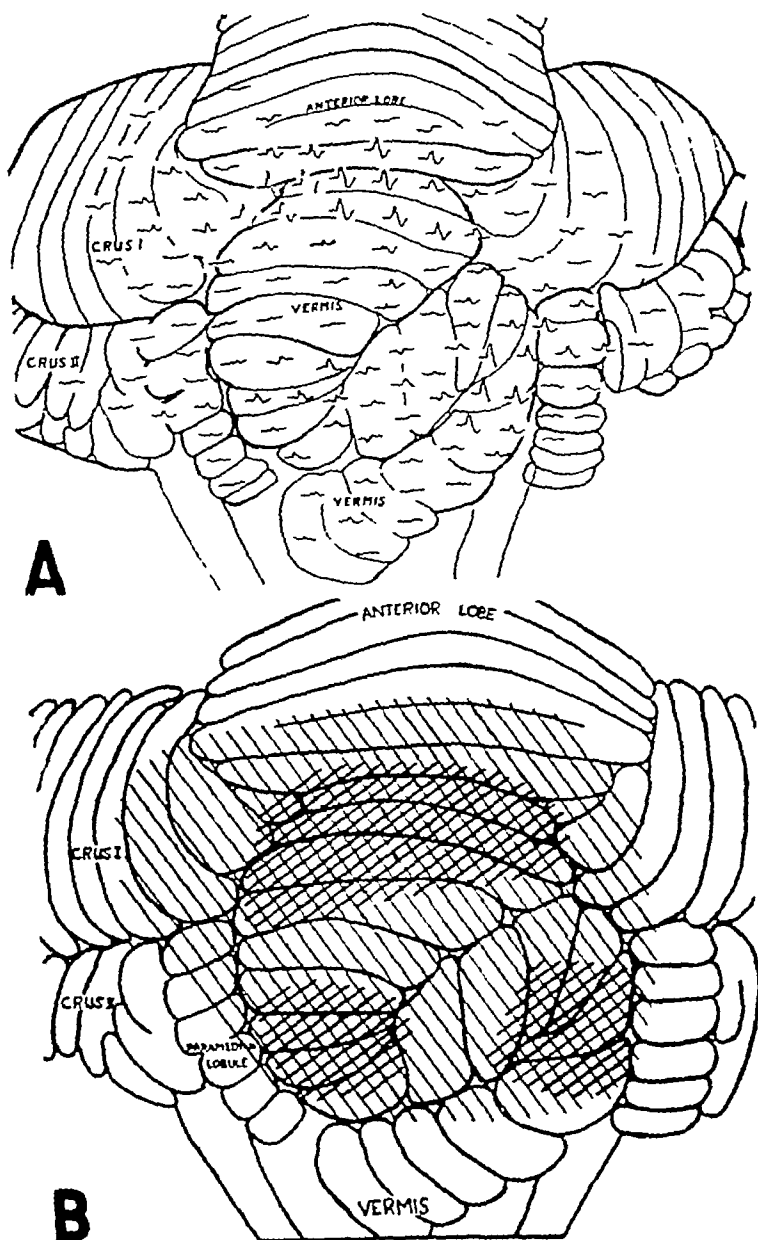


FIG. 11 Illustrations of a distribution of potentials found in some cats in response to photic stimulation of one eye. There are three zones of maximal responses. The anterior zone shown in Fig. 10 is bisected by a band of small potentials.

A Stimulation of right eye. Chloralosan anesthesia.

B Composite chart derived from results obtained in cats which showed three zones of maximal responses (cross-hatching).

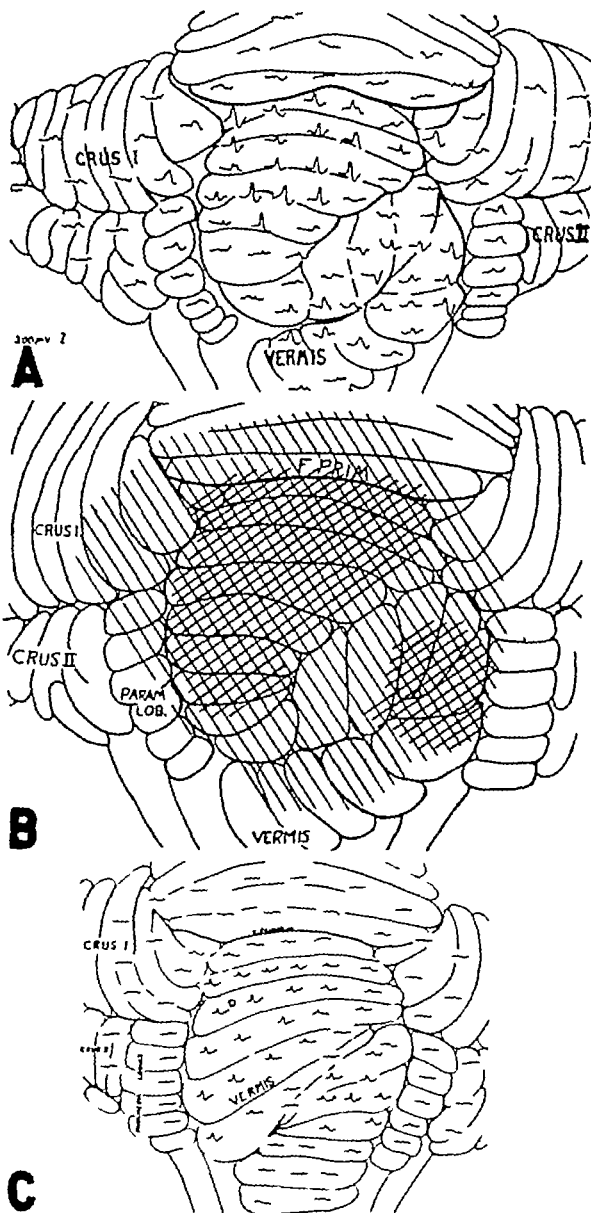


FIG 10 Illustrations of the most commonly found distribution or potentials obtained in response to flashing of light in front of one eye. There are two zones of maximal responses separated by a silent band or a zone of small potentials.

A Stimulation of right eye Chloralosan anesthesia

B Composite chart derived from results of all experiments in which two zones of maximal responses (cross-hatching) were found

C Stimulation of left eye Chloralosan anesthesia Cerebellum of unusual configuration Recording same as an experiment illustrated in A

mon form of response is made up of a surface positive component followed by a negative wave

The two or three zones of maximal responses are separated by regions or bands which are either largely silent or show only small responses which may be diphasic or have as their most prominent feature a surface negative wave. In no case did we find a visual area which was not broken by one or two such bands. When there are three zones the anterior region of large responses seen in many cats appears to be subdivided by such a band. Only small responses of one kind or another are found at the periphery of the total visual area.

In all cases there is a zone of maximal responses in the region of the second turn of the vermis. This posterior zone occupies the caudal portion of the tuber vermis, and frequently extends into the rostral folia of the pyramis, sometimes into the medial portions of the right paramedian lobule. The results of two individual experiments in which only two zones of maximal responses were found are shown in Fig 10A and 10C. It can be seen that ahead of the posterior zone just described, is a larger anterior zone which covers much of the lobulus simplex and the rostral portions of the tuber vermis. In some cases it extends, on each side, into the medial folia of crus I, a condition which is illustrated in Fig 10A. Rostrally, this zone may cross the primary fissure to invade the two or three most caudal folia of the culmen, and caudally it may involve the first three to five folia of the tuber vermis. In Fig 10B are indicated in composite form the results of some thirty experiments in which two zones of maximal responses were found. Here the total visual area is represented by diagonal lines, the zones of maximal responses by cross-hatching. A typical case of grouping of maximal responses in three zones is shown in Fig 11A. Here the anterior zone described above is divided into two parts by a band of small responses. In some experiments the dividing band may be largely silent, but more often it is the site of small responses which may be surface positive, surface negative, or diphasic. The results of experiments on some twenty cats in which three zones were encountered have been combined to form Fig 11B, which like Fig 10B, is a composite, and therefore like it represents the zones and the total area as a little more extensive than they actually are in any individual animal.

The latencies of the electrical responses evoked in the cerebellum by photic stimuli range from 40 to 50 msec and average 45 msec. Since these latencies are very much longer than those of the cerebellar responses evoked by auditory stimulation or by tactile stimulation of the forepaw, it seemed desirable to determine whether the pathway from retina to cerebellum includes a cerebral cortical relay. A conclusive answer was obtained in an experiment carried out in a cat from which all neocortex had been removed eleven months before. This animal was studied in precisely the same manner as were the normal cats of this series and, as shown in Fig 12A, yielded cerebellar responses to photic stimulation of one eye which are entirely comparable, as regards character and distribution, to those found in normal cats. It appears, therefore, that activation of the cerebellum by retinal stimulation

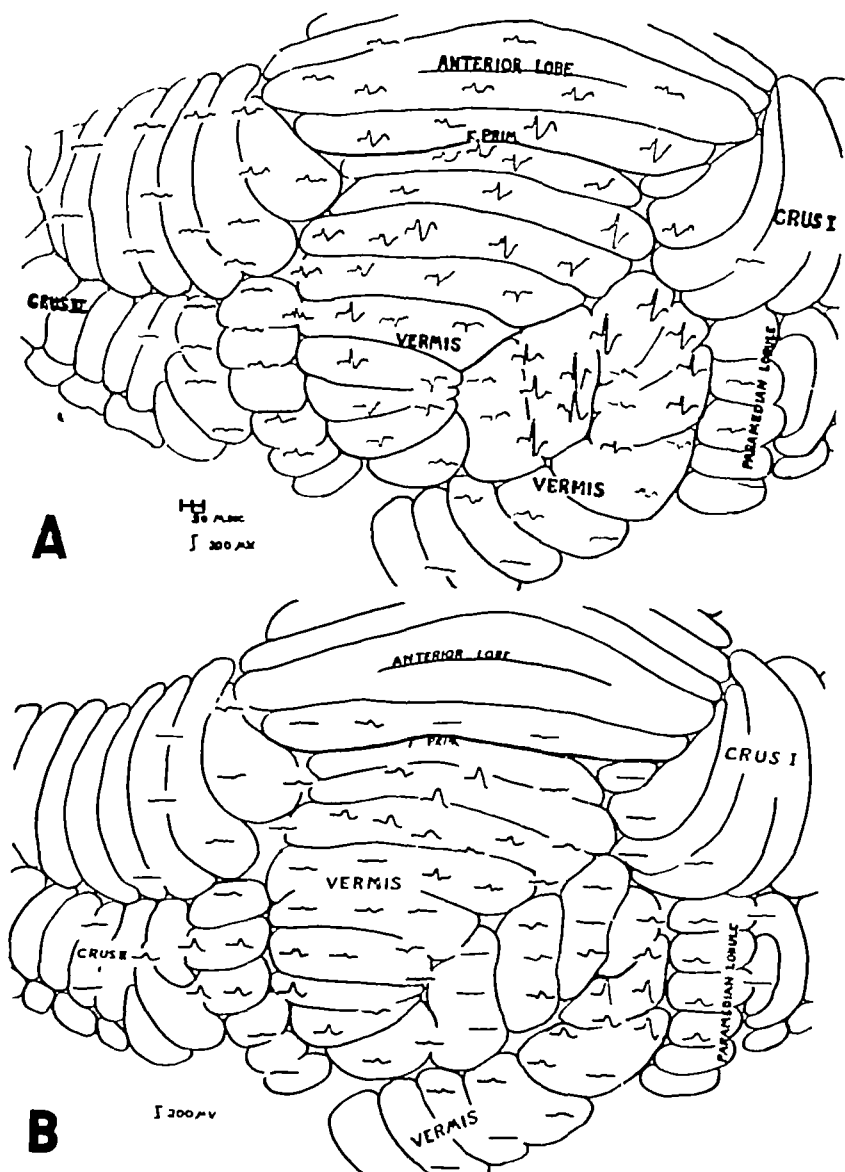


FIG 12 A Cerebellar responses evoked by photic stimulation of left eye in a cat from which all cerebral cortex had been removed eleven months before Chloralose anesthesia The distribution of the potentials and the positions of the two zones of maximal responses correspond with those found in normal animals Compare with Fig 10

B Cerebellar responses evoked by photic stimulation of left eye after bilateral removal of the extraocular muscles and eyelids Chloralose anesthesia The visual area had been mapped before these procedures were carried out In this way it was ascertained that elimination of all sense organs in the tissues removed exerted no effect on the distribution and characteristics of the visual responses

munication surely suggest that cerebellar functions are influenced by impulses which originate in tactile, auditory and visual receptors as well as by afferent discharges from proprioceptors and from the cerebral cortex. Thus they indicate that, sooner or later, present concepts of the activities of the cerebellum will have to be expanded to include the idea that impulses originating in three classes of exteroceptors and travelling over pathways which do not invade the cerebral cortex contribute to the ability of this organ of integration to exert its characteristic regulatory influence on the motor activities of certain other parts of the central nervous system.

While an exaggeration of certain responses of proprioceptive origin is an outstanding and permanent result of cerebellar removal in carnivores, there is little in the behavior of decerebellate cats or dogs to indicate that specific motor responses to tactile, auditory or visual stimuli are modified in any way except by the background of disorder in the proprioceptive sphere or by the well recognized effects of withdrawal of cerebellar influence upon motor acts which depend on the cerebral cortex. We are able to point to not more than two results of decerebellation which may conceivably be due to removal of an exteroceptive representation. Both involve the tactile system. One is the transient abolition or rise in threshold of the tactile placing reactions of the feet which was noted by Rademaker (15) in unilaterally and completely decerebellate dogs and by one of us (18) in a study on rabbits and which also has been observed recently in this laboratory in several cats and dogs during the first phase of recovery from complete ablation of the cerebellum. The other is the permanent and marked augmentation of the tactile component of the positive supporting reaction, *i.e.*, the "magnet reaction," which appears after decerebellation. One of the most universally accepted teachings of clinical neurology is that the cerebellum is not concerned with any kind of sensation, for true sensory defects have not been found among the disorders which are produced by cerebellar lesions in man. Certainly there is no clinical evidence to indicate that any cerebellar deficit is accompanied by disturbances in touch, auditory or visual perception. Yet we cannot resist wondering whether loss of the cerebellar representations of these three exteroceptive systems does not produce objective and subjective effects which are so subtle that they have escaped present methods of study.

While analysis of the characteristics of the individual potential changes evoked in the cerebellar cortex by receptor stimulation has not been our concern, brief reference to the significance of the different components of the responses may be made. The work of a number of investigators (3, 4, 13) has shown that the phases of evoked cortical potentials recorded from a pial surface when only one electrode is over the active spot may be accounted for on the basis of the assumption that impulses approaching the electrode produce a positive wave, that those which are travelling away from the electrode give rise to a negative wave, and that impulses which approach and pass under the electrode induce a change which is usually diphasic, but may be triphasic (13). In the case of the cerebellar cortex the wave form of the

does not require either the neocortex or any elements of the lateral geniculate bodies which degenerate following ablation of the visual areas of the cerebral cortex

There remains the possibility that the cerebellar responses which follow each flash of the lamp are produced indirectly through movements of the eyes or eyelids which may be reflexly evoked by the photic stimulus. If this were the case the cerebellar responses would be due to a discharge of afferent impulses from proprioceptive, tactile or pressure receptors located in or near the orbit. This possibility was examined by studying in three cats the effects of bilateral removal of the extraocular muscles and the eyelids. In each case the surgical elimination of these structures in no way altered the pattern or nature of the cerebellar responses to photic stimulation (Fig 12B). Finally, the possibility that the cerebellar responses may have been initiated by thermal stimulation of sensory endings in or around the eye at each flash of the lamp can be eliminated for several reasons. In the first place, the heating effect of a 3 watt neon bulb placed 1 to 2 inches from the cornea and flashing once per second is negligible. Then too, bringing a heated metal bar close to the eye evoked no cerebellar responses. Furthermore, electrical stimulation of the cornea or lids gave potential changes in parts of the cerebellum quite distinct from those in which potentials were evoked by each flash of the bulb. In several experiments bilateral sections of the fifth and seventh pairs of cranial nerves in no way affected the cerebellar potentials initiated by photic stimulation.

It is important to note that in our explorations of the anterior lobe we never encountered any evidence of potentials due to physical spread from responses occurring in the superior colliculi or in the visual areas of the cerebral cortex.

DISCUSSION

General considerations Unfortunately the experimental facts we have been able to bring forward do not carry with them any hint concerning their significance. Only further enquiry can provide insight as to just how and when neurally transmitted signals from tactile, auditory and photic receptors influence the activities of the cerebellum. The existing literature on the cerebellum contains few data which can in any way be correlated with our observations. In this discussion we shall mention these, make a few suggestions about the probable import of our results and devote some space to additional considerations which bear on the validity of our experimental results. To go further than this would be to indulge in futile speculation.

The relationship of the cerebellum to the proprioceptors of muscles, tendons, joints, and labyrinths has been emphasized by Sherrington's description (17) of this organ as the "head ganglion of the proprioceptive system." The other well recognized cerebellar relationship is one which has its anatomical expression in the pontile system of fibers which transmit impulses largely of cerebral cortical origin. The results described in the present com-

the most medial portions of the ipsilateral half of the anterior lobe, and the ipsilateral hindlimb, with hip near the midline, extends laterally to the hindpaw area. The head and shoulder areas appear to be coextensive near the midline. No hint of such a plan could be detected in the case of the receiving area in the paramedian lobule. It may be mentioned, however, that recent studies (22) have revealed a topographical projection in the ipsilateral paramedian lobule of the monkey.

In addition to an anatomical representation in the cerebellar cortex of different parts of the tactile surface of the body, it appears that there is also a functional representation. This is shown by the fact that the areas which receive impulses from receptors of forepaws, hindpaws and vibrissae occupy the greater part of the total representation. In other words, skin areas which possess the greatest tactile acuity (innervation density) have the most conspicuous representations in the cerebellar cortex, a situation which is not unlike that found by Woolsey, Marshall and Bard (25) in the tactile projection to the postcentral gyrus of the monkey.

In their report of an oscillographic study of a tactile projection to the cerebellum of the rat Dow and Anderson (7) state "In 26 out of 28 animals the culmen showed the largest response and in many the only surface positive potential." This is in accord with our observations on the anterior lobe of the cat as is also their finding of a surface positive potential on the lobulus simplex in about half their experiments. They also report responses due to tactile stimulation in the pyramis, but not in the paramedian lobule. In explanation of this difference in results the suggestion may be offered that in the phylogenetic development of the cerebellum there may have occurred a shift of the elements concerned in the tactile response from pyramis to paramedian lobule. Should this be the case, the method of studying projections to the cerebellar cortex by determining the distribution of evoked potentials may give, when applied to different species, valuable information in regard to the phylogenetic development of this part of the hindbrain. Dow and Anderson found no evidence of an anatomical localization in either the tactile or proprioceptive areas of the cerebellum, but they suggest that "the rat because of the small size of its cerebellum is probably not as suitable as some larger animal would be for the study of possible differences in the connections from topographically different parts of the body."

The present state of our knowledge fails to explain why one peripheral region, the forefoot or the hindfoot, is represented in two or three widely separated parts of the cerebellar cortex. It may be pointed out, however, that the physiological demonstration of the existence of these tactile receiving areas places them among the regions which receive a "spinal" projection. Many facts, of which this is one, indicate that any arbitrary distinction between paleocerebellar and neocerebellar regions is apt to lead to false impressions. Thus the tactile responses found ipsilaterally in lobulus simplex and crus I and bilaterally in the paramedian lobules confer upon these areas a distinctly spinal character which in fact has been indicated to some extent

response recorded from certain points may be complicated by concomitant activity on the bank of a nearby fissure. Nevertheless, it is true that a majority of the responses which occur in the areas we have delimited are initially surface positive or have as their chief component a positive wave, thus denoting ascending transmission in projection fibers.

The method employed by us, which involves receptor stimulation and the recording of a correlated electrical response, has been used by a number of workers (1, 12, 13, 25, 26) to map the representation of one or another sensory system in the cerebral cortex. So far as we are aware, the only previous instance of the application of this method to the cerebellum occurred in the studies of Gerard, Marshall and Saul (8). In their report of a rather general study of spontaneous and evoked electrical activity of the cat's brain these authors mention that "in three instances activity was recorded in the anterior part of the cerebellum" when sound stimuli were being used and they also state that when light was flashed in the eyes "activity was also traced to the cerebellum," but they make no further comment. Dow and Anderson's observations (6, 7) on cerebellar action potentials which they obtained in rats in response to stimulation of tactile receptors as well as proprioceptors were carried out simultaneously with and independently of ours.

No serious attempts have been made to determine the exact pathways over which impulses travel from these three classes of exteroceptors to the cerebellar cortex. Nevertheless, certain of our results indicate (i) that in no case does the pathway include a relay to the cerebral cortex, (ii) that neither the tactile nor the auditory pathways extend above the level of the inferior colliculi and (iii) that the impulses of cochlear origin pass through or close to the inferior colliculi.

Tactile representation. The most interesting aspect of this is the character of the arrangement of the areas located in the anterior lobe and adjacent folia. Here topographically different areas are related to different parts of the body surface. This is a form of anatomical localization which in general bears some likeness to that found by Adrian (1) in the cerebral cortex of the cat and by Woolsey, Marshall, and Bard (25) in the post-central gyrus of the monkey. While the most prominent and most easily determined tactile projections are related to the forefeet, the hindfeet and the vibrissae, the results obtained in many experiments suggest that most of the surface of either lateral half of the animal is projected ipsilaterally in an orderly sequence. It appears that impulses originating in tactile receptors of the caudal half of the body and the hindlimbs are received in the rostral folia of the culmen while those from the rostral portions of the trunk and from the head and forelimbs are delivered to the caudal folia of this division and to adjacent folia. One may, on the basis of our results, go so far as to suppose that the tactile surface of the cat's body is represented as stretched out flat on the anterior lobe in the following manner: the ipsilateral forelimb, with shoulder overlapping the head at the midline, extends laterally along the primary fissure to the laterally situated forepaw area, the surface of one side of the trunk occupies

changes recorded from the cerebellum cannot be due to physical spread of these electrical changes from the cochleae is shown by several facts (i) the disappearance of the potentials after destruction of the inferior colliculi, (ii) their failure to become larger as the exploring electrode approaches one or both cochleae, (iii) their abolition by section of the eighth nerves and (iv) their suppression by certain anesthetics

The features of our work which bear on the validity of the conclusion that photic retinal stimulation leads to a transmission of impulses to a definite area of the cerebellar cortex have already been presented. A few additional comments on the nature of this projection are in order. As has already been emphasized, the responses evoked by visual stimuli are not dependent on impulses which descend from the cerebral cortex. It is well known that terminals of fibers of the optic tracts pass directly to the superior colliculi, and a tecto-cerebellar pathway, which passes through the superior cerebellar peduncle, has been described in several lower mammals (9, 10, 11), it is doubtless present in the cat. Further, Rasmussen (16) has found in the cat a tract which runs from the superior colliculus to the pontine nuclei. Excitation of retinal origin might reach the cerebellar cortex by either or both of these routes. In any event it seems most likely that the visual pathway, like the auditory, involves the tectum of the mesencephalon.*

The latencies of the potentials evoked in the cerebellar visual area by the photic stimulus used in these experiments may seem unusually long. They averaged 45 msec, whereas the latencies of the auditory responses ranged from 6 to 14 msec. The contrast is evident and demands some explanation. It must be remembered that the flash of a 3-watt neon bulb placed 1 to 2 inches from the cornea constitutes a relatively weak photic stimulus. Bartley (2) has shown that in the rabbit the latency ("implicit cortical time") of the cerebral cortical response to a flash of light is lengthened as the intensity of the stimulus is decreased below a value of about 2400 candles per square foot. Marshall, Talbot and Ades (13), using a much stronger photic stimulus than we employed, found latencies of from 17 to 25 msec for the electrical response evoked in the area striata of the cat. It therefore seems probable that the long latencies of the cerebellar visual responses may be referred to the weakness of the stimulus used rather than to any peculiarity of the pathway.

SUMMARY

1 By determining the occurrence and distribution of evoked electrical responses it has been shown that there are, in the cerebellar cortex of the

* In some recent work carried out by one of us (R. S. S.) it has been found that when a point on the dorsal surface of a superior colliculus is stimulated by a weak electrical shock, evoked potential changes occur within the cerebellar visual area as delimited in this communication. It has become clear that the relation between superior colliculus and cerebellar cortex is such that distinct portions of the former are projected in a definite pattern upon the latter. Obviously these results support the conclusion that there is a true representation of the visual system in the cerebellum, and they indicate that impulses from the retina reach the cerebellum by way of the tectum.

by Dow's electrophysiological analysis of the spinal projection (5) The fact that these areas also receive pontile fibers demonstrates the futility of trying to draw distinct boundary lines between paleocerebellar and neocerebellar regions

Auditory and visual representations It is probably of some significance that the auditory and visual representations are nearly fully conterminous and overlap only slightly any part of the tactile areas This close topographical association may be related to the fact that when an animal responds to a sound it is apt to use the same muscle groups as when the effective stimulus is visual And it is also true that behavior initiated by activation of these two distance receptors involves a pattern of muscular response which is likely to be different from that set up by stimulation of "near" tactile receptors

Another similarity of the cerebellar auditory and visual projections, which sets them off from the tactile, is their greater sensitivity to the depressant action of anesthetics, especially the barbiturates Sodium pentobarbital, which was a satisfactory anesthetic for the mapping of the tactile areas, greatly reduced both the size and the distribution of the electrical responses evoked by a click In the case of the visual representation electrical responses were only rarely obtained when a surgical level of anesthesia was secured by sodium pentobarbital anesthesia When this drug was used in combination with chloralosan good responses were evocable only if the amount given did not exceed one-fourth that needed to produce, by itself, full anesthesia As indicated above, the best results were obtained when an anesthetic dose of chloralosan was administered Since negative waves, which are suggestive of corticifugal activity (4), were more prominent in experiments conducted under chloralosan, it is possible that this anesthetic not only enhances corticopetal transmission but also exerts a strychnine-like effect on synapses in the cerebellar cortex

Various control procedures were introduced to make sure that the potential changes obtained in the auditory area were due solely to auditory nerve activity set up by cochlear stimulation Most of these have been described in the section devoted to experimental results It remains, however, to dispose of two further possible sources of error The first of these is the possibility that the sound waves emitted by the loud speaker may stimulate tactile receptors of the head region or other parts of the body That such an artifact might occur is indicated by the experiments of Marshall, Woolsey and Bard (14, p 5), but several considerations exclude it as a source of error in our experiments Chief among these is the fact that there is so little overlap of the auditory and the tactile areas Also while all responses to click disappear after bilateral section of the eighth nerves they are unaffected by cutting both trigeminal nerves Further, bilateral removal of the auditory ossicles markedly reduced the amplitude of the auditory responses Another possible source of error which deserves mention is the fact that the click doubtless produces microphonic action in the cochlea That the potential

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cat, distinct areas which receive nerve impulses from tactile, auditory and visual receptors

2 The receiving areas for impulses of tactile origin are located in three parts of the cerebellum. In each lateral half of the anterior lobe and in adjacent folia there is a projection of the ipsilateral half of the body surface in which the feet and the vibrissae are the parts most conspicuously represented. The forefoot and hindfoot of one side are represented in both paramedian lobules, the forefoot having a stronger contralateral representation than the hindfoot. In the rostral area (anterior lobe and adjacent folia) the tactile representation shows an anatomical localization in the sense that different zones are related to different parts of the body surface.

3 The auditory area is largely restricted to the lobulus simplex and the tuber vermis. In most cats it extends somewhat into the posterior folia of the culmen, the anterior folia of the pyramis, the medial portions of crus I on each side and both paramedian lobules. The responses evoked by auditory stimuli are not affected by decerebration at an intercollicular level. They are abolished by section of the eighth nerves or destruction of the cochleae or removal of the inferior colliculi. They are not altered by section of the fifth nerves.

4 The visual area overlaps the auditory and in most cats the two areas are continuous. The cerebellar responses to photic stimuli are not modified by bilateral section of the fifth and seventh cranial nerves or by removal of eyelids and extraocular muscles on both sides. They are unaffected by removal of all cerebral neocortex and depend on a pathway which probably involves the superior colliculi.

5 Full anesthetic doses of sodium pentobarbital appear to have little effect on the tactile responses, but they markedly depress the auditory and completely abolish the visual. The auditory and visual areas are most clearly revealed when chloralose is used as the anesthetic.

6 It is suggested that cerebellar functions are influenced by tactile, auditory and visual stimuli as well as by impulses from proprioceptors and from the cerebral cortex.

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SPREADING DEPRESSION OF ACTIVITY IN THE CEREBRAL CORTEX*

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THIS STUDY originated in an attempt to secure more data for the understanding of the cortical electrogram which occurs in "experimental epilepsy," and of the conditions in which it is brought forth by electrical stimulation. Early in the development of the study an interesting response, elicited by electrical stimulation, was noticed in the cortex of rabbits. The distinctive feature of this response was a marked, enduring reduction of the "spontaneous" electrical activity of the cortex. We have endeavored to define experimentally some of the characteristics of this response.

METHODS

Almost all of the experiments were performed on rabbits. Dial narcosis was used routinely. The drug (dial-urethane solution, Ciba) was administered by intraperitoneal injection, in doses varying from 0.55 to 0.75 cc per kg of body weight. Nembutal was used occasionally, and chloralose, or ether were employed in several experiments. A tracheal cannula was always inserted. The animals were laid prone, with the head supported by a Czermak holder, and one or both cerebral hemispheres were widely exposed. Seven major cortical regions, according to Rose (37) are represented in the dorsolateral part of the hemisphere, investigated in this study: *praecentralis granularis*, *praecentralis agranularis*, *postcentralis*, *parietalis*, *temporalis*, *occipitalis*, and *retrosplenialis granularis*. The lead-off and stimulating electrodes were applied to the surface of the pia-mater. They were, as a rule, fine chlorided silver wires, with a small bead at the tip. Electrodes of the Ag-AgCl-agar-Ringer type, with a cotton tuft, were also used occasionally for recording. Electrical stimulation of the cerebral cortex was obtained with the "tetanizing" current of a Harvard induction coil, or with single condenser discharges delivered through a key operated by hand. The interelectrode distance, for stimulation, was about 1.5 mm in all cases. Amplification of the bio-electrical phenomena of the cerebral cortex was obtained with a six-channel Grass ink-writing oscillograph. The input of each pair of electrodes was on push-pull. The animal was, as a rule, grounded through the tracheal cannula, but in the experiments in which single condenser discharges were applied to the cortex while electrograms were being taken, a "Wagner ground" was used, in order to control the stimulus artifact. The balancing circuit consisted of a 10,000 ohm potentiometer shunted across the stimulating electrodes and with the center tap grounded.

Several experiments were carried out on pigeons, under nembutal narcosis, and only a few on cats. In the latter, the brainstem was transected at the intercolumellar level, while the animals were under ether. The observations were made later, after elimination of the anesthetic. This preparation has been described by Bremer (11), who designated it the "cerveau isolé." Nembutal was also used in cats.

RESULTS

Unless otherwise stated, the results described were obtained on rabbits under dial narcosis.

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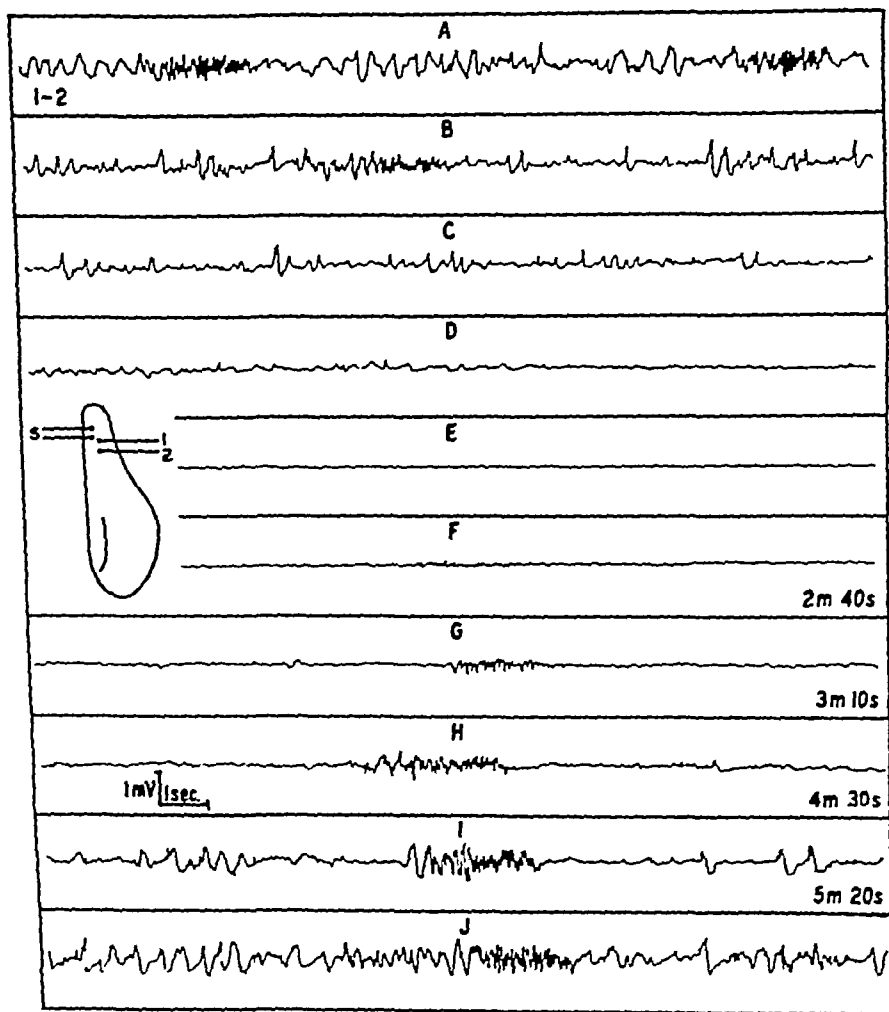


FIG 1 Depression of the spontaneous electrical activity at the region stimulated. Stimulating (S) and recording (1-2) electrodes arranged as shown in the inset. A Before stimulation. B to E. Continuous record starting less than 1 sec after the end of stimulation. F to I. Records, corresponding to the times noted, after stimulation. J. Complete recovery, 5 min after I.

the region stimulated, so that increasingly distant parts of the cortex were successively involved, i.e., they exhibited also a marked, enduring depression of their electrical activity. Figure 3 illustrates this spread. The time after stimulation at which each of the representative steps was taken, shows the slow rate of travel of the response. A response started near the frontal pole might take more than 5 minutes to reach a region near the occipital pole. From the stimulated region, the wave of depression spread out to almost all of the cortex exposed in the usual preparation. The only exception noted was

Different, regional patterns of the so-called "spontaneous" electrical activity of the cerebral cortex were clearly recognizable. These patterns resemble the "Feldeigenstromtypen" described by Kornmüller (30, 31)

DEPRESSION OF ACTIVITY

A Depression of "spontaneous" electrical activity of cerebral cortex, caused by electrical stimulation Following a brief period of repetitive electrical stimulation of the cerebral cortex (1 to 5 sec of "tentanizing" current from a Harvard induction coil), a response consisting of a marked, enduring depression of its "spontaneous" electrical activity was observed. This type of response was obtained when the stimulation was below threshold for the production of any electrical "after-discharge."

If the lead-off electrodes were placed in the close vicinity (1 to 2 mm) of the stimulated region, a depression, in progress or already well established, could be seen immediately after the period of stimulation, or else it could be noticed only after an interval (10 to 20 sec), during which the spontaneous electrical activity of the cortex did not show any alteration. This "latency" for the appearance of the depression depended on the strength and duration of stimulation. It was longer when the intensity of the shocks was weak or when the period of stimulation was brief. Figure 1 illustrates a typical response. The arrangement of the electrodes is shown in the inset. In A the spontaneous electrical activity is seen. In B, C, D, and E, a continuous record, starting less than 1 sec after the stimulation, the progress of the depression is seen. During the time (about 15 min) between E and F, the electrogram was similar to that in E. The gradual return to the original spontaneous activity is seen in F, G, H, I and J, which are representative steps of its long course. The duration of this depression was variable. The first signs of "recovery" were often observed earlier than in the response illustrated, or they might appear later, a marked depression then persisting for a few minutes. Usually, in light narcosis the first signs of recovery were noticed early, and the recovery was rapid. In those regions in which the pattern of the spontaneous electrical activity is characterized by recurring "bursts" of discharges (for instance, the area precentralis granularis, of Rose), these bursts typically constituted the first signs noticed in the slow recovery. The voltage of the successive bursts gradually increased, and the bursts became more frequent. As a rule the slower waves between the bursts did not appear until later. In the recovery of the area striata, groups of the characteristic, slow waves separated by periods without them were seen for several minutes before the original pattern was reconstituted.

As a rule, the depression was seen merely as a progressive diminution of the background of potential fluctuations, without any sign of specific patterns. Occasionally, however, the initial pattern became simplified, and a more regular rhythm predominated until the depression became complete (Fig. 2).

B Spread of depression Simultaneous records from several pairs of electrodes showed that the depression spread out slowly, in all directions, from

When the recovery of the regions near the stimulating electrodes was rapid, the spontaneous electrical activity might have returned more or less to "normal" there, while the response was starting in distant parts (Fig 5) The spread of the depression within a given cytoarchitectonic area was progressive, *i e*, the spontaneous electrical activity in these units did not change

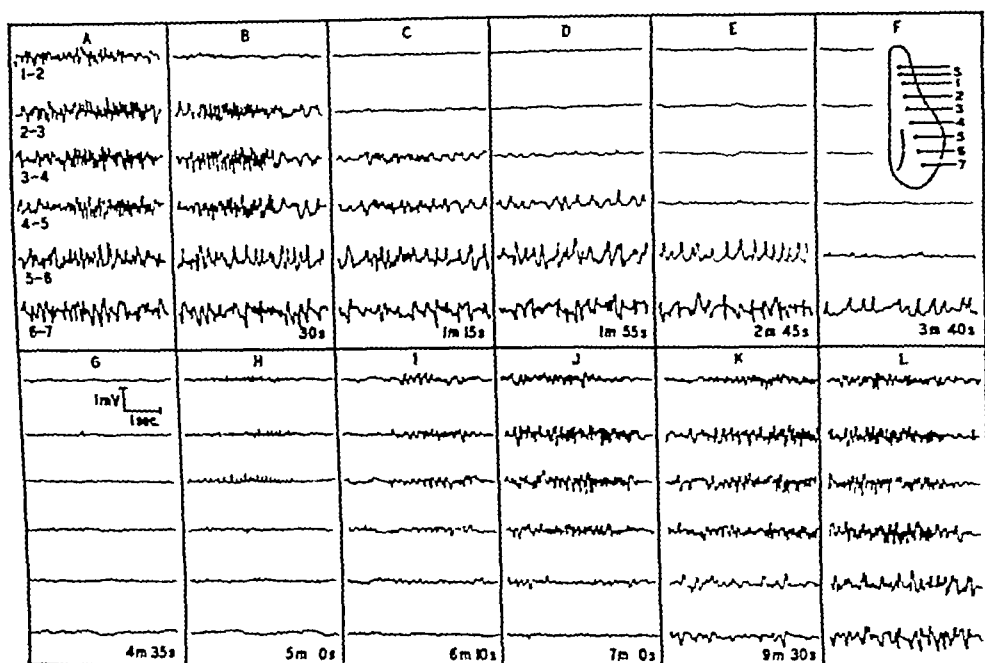


FIG 3 Gradual spread of depression. Electrodes arranged as shown in the inset A Before stimulation L 7 min after K

In this and the following figures, when a strip is timed, that time indicates the interval from the end of the stimulation period. Upward excursions in the records denote negativity of the more frontal electrode (lower number) with respect to the more occipital electrode (higher number). Unless otherwise mentioned, the stimuli were induction shocks at "tetanizing" frequency, applied for 3 to 5 sec through electrodes S.

simultaneously all over the area, one part might still be active while another portion was depressed. After a wave of depression had spread over the cortex, it took several minutes (5, 10, or even more) before the spontaneous electrical activity returned to normal in all regions. A second spreading response could be elicited earlier than this, but not until the recovery was well advanced at the region stimulated.

C Mechanical stimulation Spreading depression of the electrical activity could also be elicited by mechanical stimulation of the cerebral cortex. One or a few light touches with a small glass rod evoked it easily. These mechanical stimuli caused only a slight compression of the tissue, without any visible structural damage. The point touched could not be distinguished later from the neighboring cortex. Depression then appeared at the stimulated region.

a small region, medial to the parasagittal sulcus. This region corresponds to the area retrosplenialis granularis dorsalis (Rsg β) of Rose (area 29d of Brodmann [12], area u of Droogleever Fortuyn [18]). The spontaneous electrical activity in this area did not show any appreciable decrease while the depression spread along adjacent regions (Fig. 4)

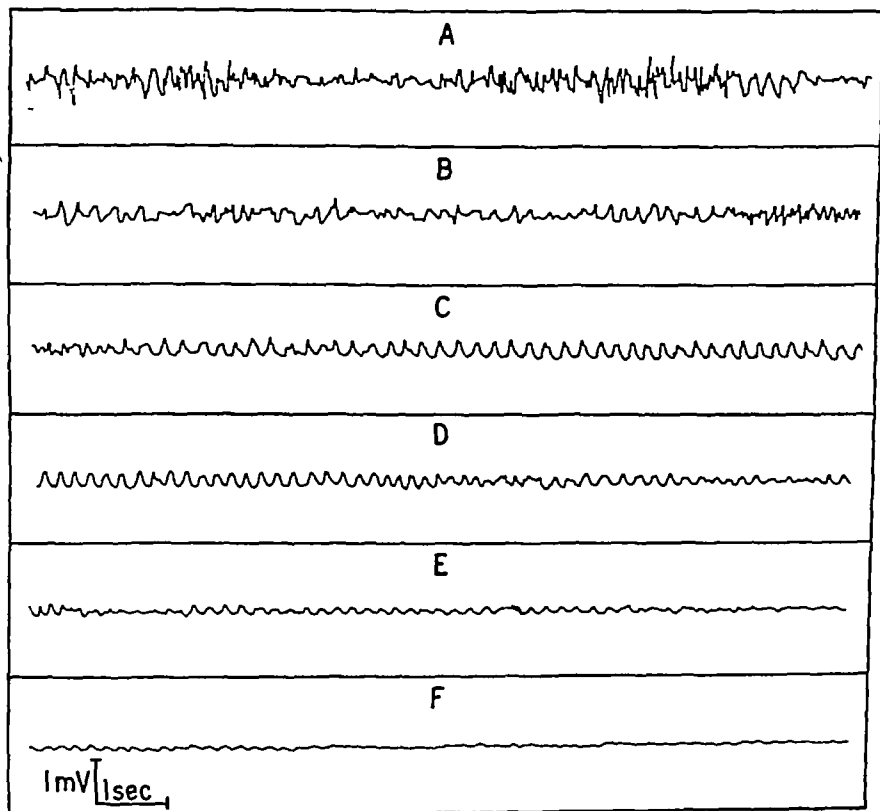


FIG. 2. Relatively unusual mode of onset of depression. Stimulus and record as in Fig. 1. A to F. Continuous record, starting immediately after stimulation.

At any one place on the cortex, not in the close vicinity of the stimulating electrodes, the depression of the spontaneous electrical activity developed gradually. About 20 seconds to 1 minute elapsed from the moment that the first signs of depression were noticed to the time when depression was maximal. During this time, as a rule, a mere progressive diminution of the background of potential fluctuations was seen. Occasionally, however, as in the region stimulated, a more regular rhythm predominated until the depression became complete. The electrical inactivity then persisted for a variable time, as in the region stimulated. Here again, the recovery showed a slow course, and usually it took several minutes for the electrical activity to return completely to normal.

for instance the posterior part of the area striata, were more variable in different animals. A typical response would often be elicited with weak stimulation, but sometimes stronger stimuli were required than those effective in the frontal regions. In certain cases, a stimulation was required that was strong enough to produce a brief "after-discharge" in the close vicinity of

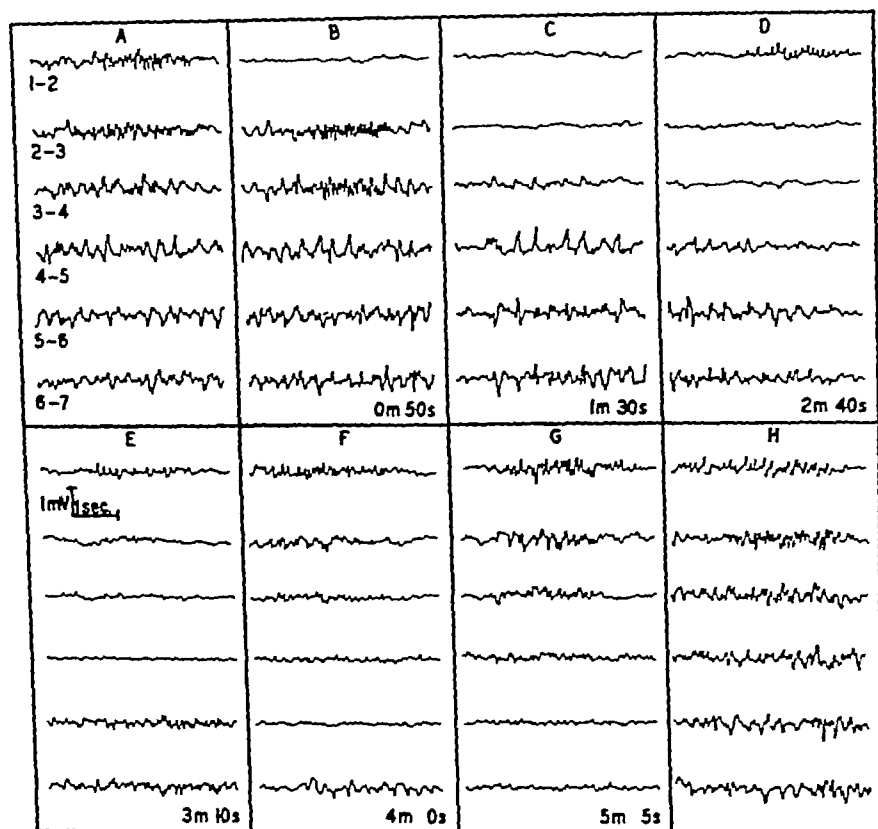


FIG 5 Rapid recovery of spontaneous activity in regions near the stimulating electrodes. Electrodes arranged as in Fig 3. A Before stimulation. H Complete recovery, record taken 3 min after G.

the stimulating electrodes. This brief "after-discharge" was confined to this region, and was followed, usually after an interval of several seconds, by depression of the electrical activity which then spread out in all directions and ultimately reached the frontal pole. Finally, in a number of animals a widespread wave of depression could not be elicited at all by stimulation of the more posterior portions of the area striata. A brief depression of the electrical activity could be seen at the stimulated region, and sometimes it spread out for only a few millimeters. While it was sometimes difficult to initiate a spreading wave of depression from the area striata, it was always possible,

after a variable latency (10 to 40 sec) It progressed and spread over the cortex, as described When electrodes were being placed on the pial surface, responses were occasionally elicited by this mechanism

D *Regional differences* Stimulation of the frontal regions of the hemi-

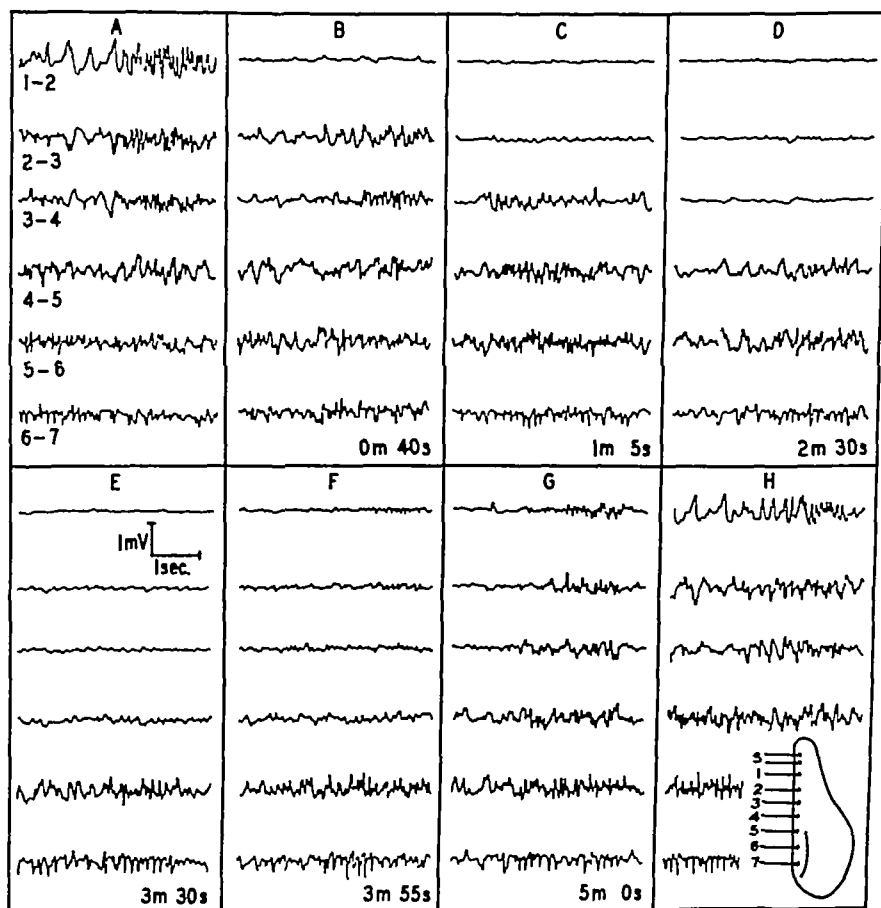


FIG 4 Absence of spread of depression to the area retrosplenialis (electrodes 6 and 7, see inset) A Before stimulation H Recovery, 6 mm after G (see legend of Fig 3 for additional explanation)

sphere with appropriate weak intensity was followed invariably by a typical response The wave of depression traveled throughout the exposed cortex, with the exception noted above Responses were also easily obtained when the stimuli were applied to regions about midway from the frontal to the occipital pole The depression would then spread out slowly in all directions, and would reach the regions near the two poles of the hemisphere at about the same time The results of stimulation of regions near the occipital pole,

movements of the vibrissae—were easily recorded. In Fig. 6 I is illustrated a typical response to a light tap on the forefoot. The responses consisted of a brief potential wave, positive with respect to an electrode outside the responding area, always clearly recognizable, followed immediately in most in-

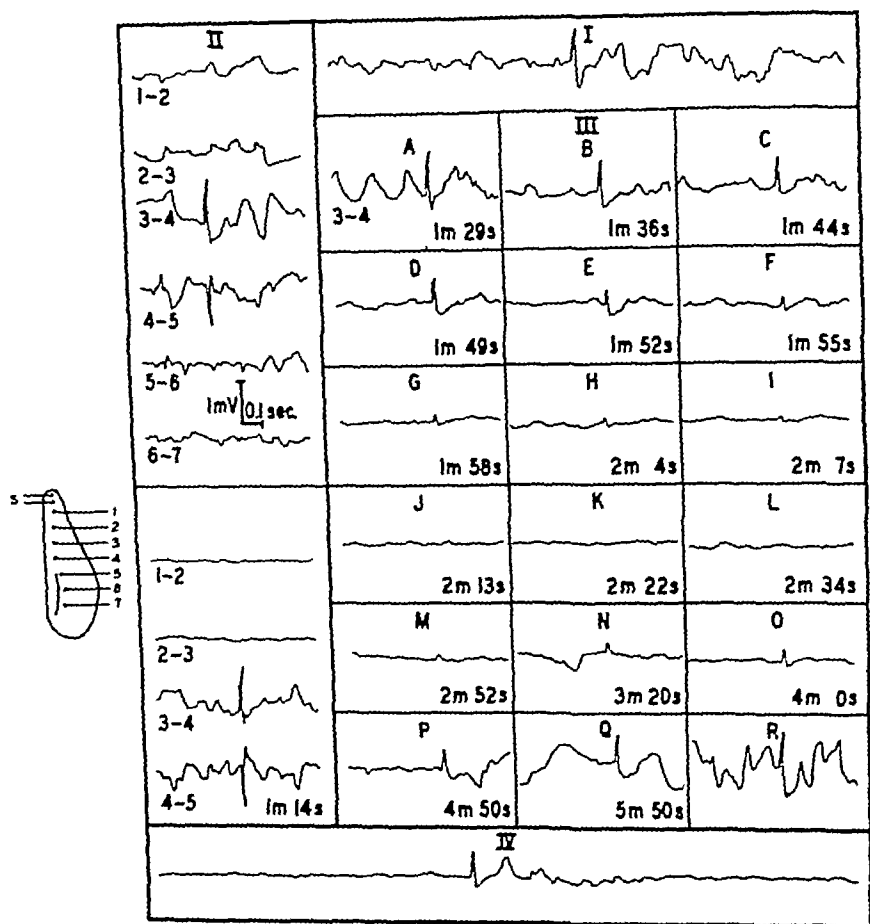


FIG. 6 Depression of responses to touch. Electrodes arranged as shown in the inset. I A typical response. II Upper section simultaneous records, showing the response localized to electrode 4. Lower section record taken 1 min 14 sec after stimulation (the depression has reached electrode 3, the responses, at 4, are unimpaired). III Continuation of II, showing the depression of the responses when the process reaches electrode 4 (R, complete recovery, was taken 8 min after Q). IV A response, during the recovery of depression, showing the complexity of the events elicited by each tactile stimulus.

stances by a negative excursion, more variable in amplitude and duration. There were also later components, variable, and difficult of description because of the interfering spontaneous excursions. If the stimulation was repeated about every 3 seconds, a series of quite similar responses was ob-

however, to involve this area in a response, *i e*, to have its electrical activity depressed, by stimulation of the more frontal regions of the hemisphere

In regions of the cortex other than the area striata, if the strength of the stimulus was sufficient to produce a local brief "after-discharge," this activity was followed by depression which then spread out as described. Although the depression was marked all over the cortex, it was as a rule somewhat more conspicuous and enduring at the regions in the anterior half of the hemisphere, especially the more frontal ones

If only minimal effective stimuli were used, which were well below threshold for the production of any electrical "after-discharge," the response at any one particular region of the cortex had the same time course and magnitude whether the stimuli were applied near or far, and regardless of the region stimulated. Two waves of depression could be started simultaneously at distant regions of the cortex. They then spread in the usual manner, so that two waves might travel in opposite directions to reach an intermediate cortical region

E Mechanism of spread The spread of depression could be delayed or entirely prevented from reaching some part of the cortex by applying for a few minutes in appropriate positions on the pial surface strips of filter-paper soaked with a solution of cocaine hydrochloride (10 per cent). Damage to a narrow band of tissue by application of a hot wire, or a superficial cut, also interfered with the spread of the depression. This evidence suggests that the spread of the depression is a cortical process, *i e*, it takes place within the gray matter, or perhaps it also involves the underlying white, but it probably does not require a contribution from sub-cortical centers

F Elicitation of depression in opposite hemisphere The depression of the electrical activity could also be elicited in the opposite hemisphere. In this case, the response appeared first at the region symmetrical to the one stimulated, and spread out from there to the rest of the hemisphere. If very weak stimulation was used to start a response, it usually did not cross. No change was then noticeable in the spontaneous electrical activity of the opposite hemisphere, throughout the time the depression was spreading in the stimulated side. If the strength of the stimulation was increased a little, the crossed response was obtained, but at the symmetrical region the "latency" for the establishment of the depression was longer than it was at the stimulated region, so that the spreads in the two hemispheres were not coincident. In the crossed side it started a little later, and, as a rule, at any given time after stimulation, it had covered a smaller area than that reached on the stimulated side. The crossing was much more easily obtained by stimulation of the anterior half of the hemisphere, especially the more frontal areas

G Depression of sensory responses (1) *Responses in the somatic sensory cortex*—The area in the rabbit's cortex, which receives the afferent impulses relayed from tactile and other receptors of the body and limbs has been mapped by Adrian. (2) *Cortical responses to mechanical stimulation of the somatic receptors*—light taps on the forefoot, the hindfoot, or the lips, or

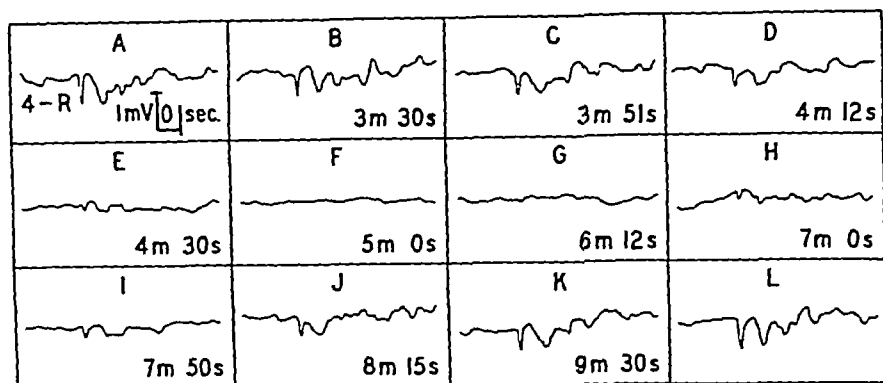


FIG 7 Depression of optic responses. Electrodes arranged as in Fig 3. R Indicates a reference, extracortical electrode. A Typical response, before stimulation. L Complete recovery, record taken 4 min after K.

entire area was involved, and the gradual recovery of the responses occurred in the same sequence as the depression.

H *Depression of responses to electrical stimulation of cortex* (1) *Responses in the vicinity of a region stimulated with single shocks*—A complex response starting with a distinct potential wave, during which the electrode proximal to the stimulated region went positive with respect to the distal one, was recorded with the electrodes arranged in the manner shown in the diagram accompanying Fig 8. While a series of single shocks was being delivered, about one every three seconds, a spreading depression was started at a distant region. The depression and recovery of the responses, as the wave of depression passed through the recording region, are shown in Fig 8, with representative steps taken at different times. The series has the same general features as those presented for the sensory responses.

(2) *Responses in contralateral hemisphere*—These have been described by Curtis (15, 16). The responses were evoked by single shocks applied to the pial surface on one side, and were recorded from the opposite hemisphere at the region symmetrical to the one stimulated. They consisted of an initial

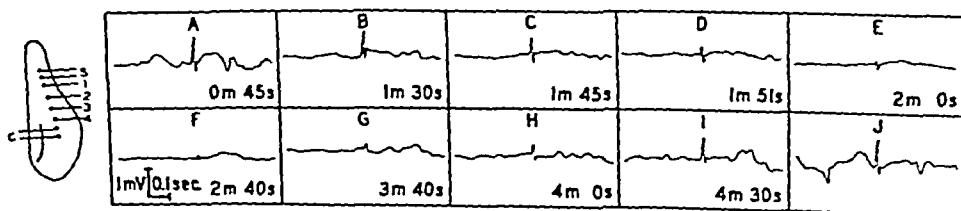


FIG 8 Depression of the responses to electrical stimulation of the cortex. Responses recorded in the vicinity of a region stimulated with single shocks. Single shocks (condenser discharges) delivered through electrodes C. Responses recorded from electrodes 3 and 4. The response illustrated in A was similar to those before the stimulation eliciting depression. J Complete recovery, record taken 8 min after I.

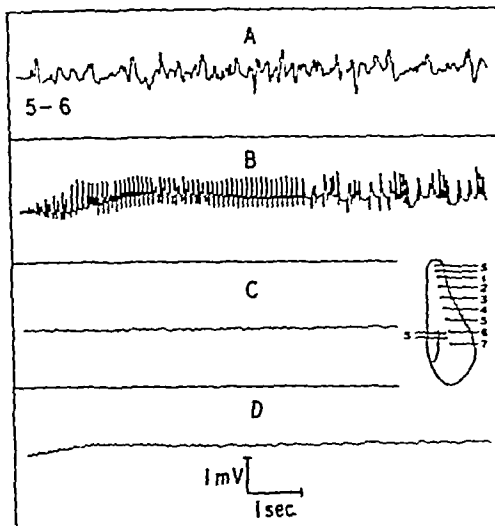
tained While a series of tactile stimuli was thus delivered continuously, if a spreading depression was started at any distant region of the cortex, the following results were observed The sensory responses, much as the spontaneous electrical activity, remained unchanged until the advancing front of the depression wave reached the region in question Then, both the sensory responses and the spontaneous activity declined gradually and approximately in parallel A few small sensory responses were usually obtained after the spontaneous excursions had ceased to be noticeable, but for a short time, at the peak of the depression, the responses could no longer be recognized The sensory responses reappeared relatively early in the recovery period and increased gradually, over an interval of several minutes, to reach their initial amplitude at about the same time as the spontaneous activity Figure 6, II and III, illustrates a typical observation with a number of representative steps The responses, in the progress of the depression, might be reduced to single positive waves, but often a small negative phase followed even when the positive component was greatly reduced Late in the recovery, while the spontaneous electrical activity was still considerably reduced, the complexity of the sensory responses was clearly manifest (Fig 6 IV) If, early in the depression, the amplification of the electrogram was increased, a sensory response could still be recognized with ease, when it had become barely noticeable with the usual amplification At the peak of the depression, however, no wave could be distinguished as a separate response from the small oscillations of the background

Characteristic sensory responses could be elicited in one hemisphere throughout a depression of the opposite hemisphere The responses to electrical stimulation of a spinal afferent nerve were also examined The progress of the depression and the course of the recovery of these sensory responses, elicited by single shocks applied at intervals of about 3 seconds to the contralateral ulnar nerve, were similar to those described for the responses to mechanical stimulation of the receptors

(2) *Optic responses* —These responses have been described in detail by Bartley, Bishop and O'Leary (5-10) The responses of the area striata were evoked by illumination of the eye The contralateral retina was illuminated for about 2 or 3 seconds, every 5 or 6 seconds, and "on" or "off" effects were observed, or else a brief flash of light was used, repeated at intervals of about 3 seconds The depression and recovery of the optic responses occurred again in a manner similar to that described for the responses in the somatic sensory cortex The electrograms decreased in amplitude but, as a rule, remained complex until they were well depressed Figure 7 illustrates a typical observation Owing to the large extent of the responding area, the progressive spread of the depression of the responses inside the area striata was easily observed Three or 4 electrodes were placed on this area, in line along the direction of spread of a wave of depression started at some distant region When the wave reached the first electrodes the responses were depressed there while still unaffected at the remaining ones Subsequently the

way as already described for the sensory responses to touch Figure 9 I illustrates a typical experiment If, while a series of single shocks was being delivered, a wave of depression was started in the *stimulated hemisphere* and did not spread to the opposite side, the results were as follows When the depression reached the region of the stimulating electrodes, the responses recorded from the opposite hemisphere became progressively smaller and after an interval of half a minute or so they were not recognizable They reap-

FIG 10 Depression of tonic-clonic responses A Spontaneous activity B Tonic-clonic response following strong stimulation through electrodes S C Depression after appropriate weak stimulation through electrodes S' D Absence of tonic-clonic response when the same stimuli as in B were reapplied during the depression



peared a little later and gradually grew to reach their original magnitude after a few minutes In this case, as opposed to the previous one, depression and recovery of the responses occurred while no change was noticeable in the spontaneous activity Figure 9 II illustrates characteristic results

(3) Responses of "self-sustained" type (38) elicited by strong repetitive electrical stimulation (experimental epilepsy) —At the peak of a wave of depression, a train of stimuli which previously was effective in bringing forth a self-sustained response, might have little or no immediate effect Figure 10 illustrates an experiment The electrodes were arranged as shown in the inset A brief period of repetitive electrical stimulation of adequate intensity, from the coil S, produced the response shown in B This activity in the region of the stimulating electrodes was followed by a wave of depression which spread throughout the cortex After complete recovery, another wave of depression was started, this time from the coil S' and with weaker intensity of stimulation, well below threshold for the production of any after-discharge About 3.5 minutes later, the wave reached the region of the electrodes of the coil S When the depression was maximal there, a second stimulation from this coil was applied The strength of the shocks and the dura-

potential wave, positive with respect to an electrode outside the responding area, always clearly recognizable, followed immediately in most instances by a negative wave, variable in amplitude and duration, and by other later components. With electrodes arranged as shown in the diagram (Fig 9), a

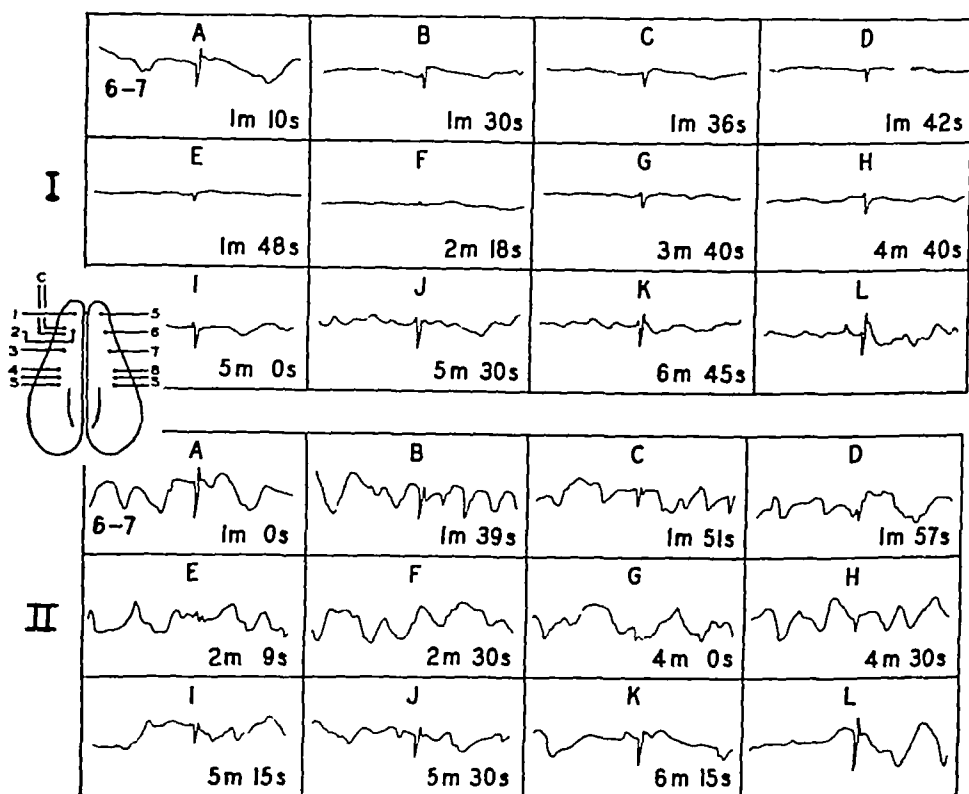


FIG 9 Depression of the responses elicited in the contralateral hemisphere by single shocks applied to the pial surface. Single shocks (condenser discharges) applied to *left* cortex, through electrodes C. Responses recorded in the *right* hemisphere, from electrodes 6 and 7. I Depression spreading over *right* hemisphere (elicited through electrodes S, right). The response in A is similar to those recorded before the stimulus was applied at S L. Complete recovery, 7 min after K. II Depression spreading over *left* hemisphere (elicited through electrodes S, left). The response shown in A was similar to those before the stimulation eliciting depression. L 5 min after K.

spreading depression was started in either hemisphere, and its unilateral spread was ascertained. The single shocks were repeated about every 3 seconds, and a series of quite similar responses was obtained. While the single shocks were being delivered, a wave of depression was evoked and spread only within the hemisphere where the crossed responses were being recorded. When the depression reached the observed region the responses, together with the spontaneous activity, were depressed in much the same

area and hence the spikes were present in a relatively large territory, or if the effects were too intense, then it was not possible to elicit a wave of depression which would travel within the region

(2) *Eserine and acetylcholine* —Many points concerning the complex effects of local applications of these drugs on the cortex of the rabbit are not well known. A study of Chatfield and Dempsey (14) revealed the existence of regional differences in the effect of local applications of these drugs to the cat's cortex. Miller, Stavraky and Woonton (35) studied the effects on rabbits but did not report regional differences. In the present observations no attempt was made to obtain an accurate description. Local applications of eserine (a small piece of filter paper, about 1.5×3 mm, soaked in 0.5 per cent eserine salicylate) resulted in most instances in a decrease of the voltages of the spontaneous activity at the treated site. This modified activity was depressed, and later recovered, when a wave of depression, started at some distance, spread past the treated region. Application of acetylcholine (0.5 per cent acetylcholine chloride), in a similar manner, to a region previously treated with eserine, led promptly to the appearance of fast, large potential waves (spikes), occurring in bursts, or, sometimes, in a more or less continuous succession. A rapid, low voltage activity was also induced. These excursions could be depressed when, following adequate stimulation a wave of depression was started at a distant region and spread to the area treated with the drugs. The depression was, as a rule, brief, electrical activity promptly reappearing. If the drugs were applied to a large extent of cortex, or if the effects were very intense, the excursions could not be depressed.

ACTIVE ELECTRICAL PHENOMENA WHICH MAY OCCUR DURING DEPRESSION OF SPONTANEOUS ACTIVITY

Electrical activity of various types, but always clearly different from the spontaneous activity, was often observed during the period of depression in various cortical regions. This activity was usually more prominent when the hemisphere had been exposed for some time or when it had been repeatedly stimulated, particularly if strong stimuli had been used. Although activity of this sort might appear in any region of the cortex, it was, as a rule, less intense and less frequently seen in the more frontal regions. Some of the potential waves that occurred during the period of depression of the spontaneous activity had characteristic features and could be easily identified.

A *Slow potential waves* Large, slow, negative waves were the most common of the several types of activity. In the course of each wave one of the recording electrodes became negative with respect to others placed on the pial surface, 1.5 to 3 mm away. Thus, in Fig. 12A, electrode 5 became negative with respect to electrodes 4 and 6. No potential change was recorded between electrodes 4 and 3 or 6 and 7. The event is therefore localized to cortical elements in the neighborhood of lead 5. Because of their large amplitude and long duration, as compared to the excursions of the spontaneous activity, these waves were markedly distorted in some of the electrograms. They were

tion of the period of stimulation were the same as before. The electrogram reproduced in D shows that no activity resulted.

I Depression of activity induced by drugs (1) *Strychnine*—As is well known, application of strychnine to a region of the cerebral cortex leads to the local appearance of large, recurring, composite potential fluctuations. These are usually referred to as the "strychnine spikes." In a number of

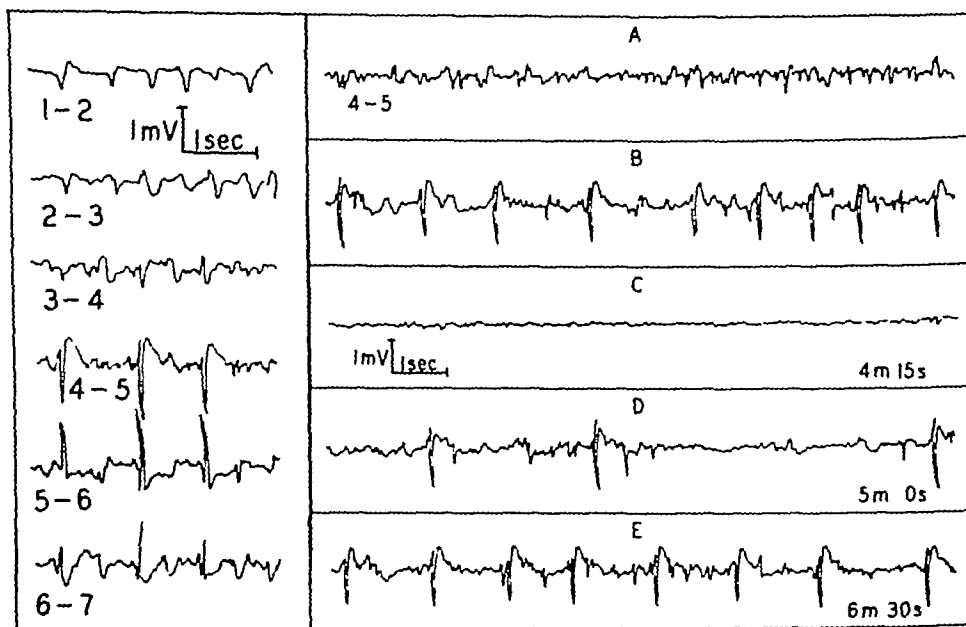


FIG. 11. Depression of the activity induced by strychnine. Electrodes arranged as in Fig. 3. Filter paper soaked in 0.1 per cent strychnine sulphate applied near electrode 5. A: Spontaneous activity. B: Strychnine spikes, record taken 7 min. after application of the drug (see also, at the left, the simultaneous records from all pairs of electrodes). C: Depression of the strychnine spikes (the depression was elicited by electrical stimulation through electrodes S). D and E: Recovery.

experiments a small piece of filter paper (about 1.5×3 mm) soaked with a 0.1 per cent solution of strychnine sulfate was applied to the cortex close to one of the recording electrodes. Some minutes later, after the effects of the drug had developed, a wave of depression was started at a distant region. The spread past the strychninized region was followed with several pairs of electrodes. It occurred in the same manner as it did before the drug was applied. When the wave reached the treated region, the strychnine spikes, which were recurring at short intervals, became more widely spaced and promptly disappeared. None were recorded during a variable period (20 sec to 1 min). They then reappeared, first separated by long intervals and later (about 1 to 3 min) with the frequency which prevailed before the depression. Figure 11 illustrates a typical observation. If the drug was applied to a wide

An individual slow wave could be recorded from several electrodes placed within the cortical region where they were occurring. As the electrograms in Fig. 12C show, the negativity did not develop simultaneously in all places. First, electrode 3 went negative with respect to electrodes 2 and 4, and later electrode 4 went negative with respect to electrodes 3 and 5. This indicates

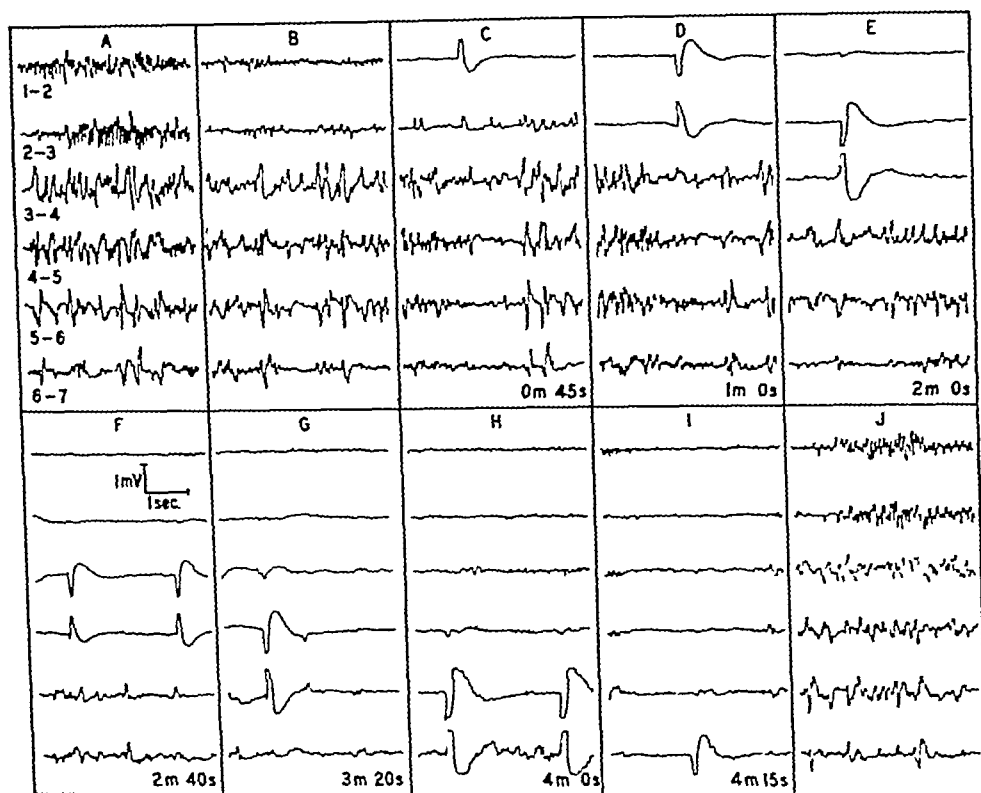


FIG. 13 Slow negative waves occurring successively in different regions as the peak of the wave of depression successively reaches these regions. Electrodes arranged as shown in the inset of Fig. 3. A Before stimulation. B Immediately after stimulation. J 10 min after I (see legend of Fig. 3, for additional explanation).

that the negativity originated at a certain focus, and spread from this focus for a few millimeters. The electrograms in Fig. 12D illustrate in addition that there was not a fixed focus, inside the region, from which the waves always started when they were recurring. In the second wave, Fig. 12D, electrode 4 became initially negative, and, in the third wave, it was electrode 3 which became negative first. In the first wave, the negativity developed almost simultaneously at electrodes 3 and 4, no large deflection was recorded between these two electrodes, while 3 was negative with respect to 2, and 4 was negative with respect to 5. Although the active zone was moving over

distorted in amplitude because correction of this distortion would have rendered the spontaneous excursions so small that changes in them would have been difficult to recognize. They were also noticeably distorted in phase when the time constant of 0.15 sec. was used for the amplifiers. With the longer time-constant of the amplifiers (0.5 sec.) and with reduced amplification an adequate record could be obtained. Such a record is shown in Fig. 12B, and

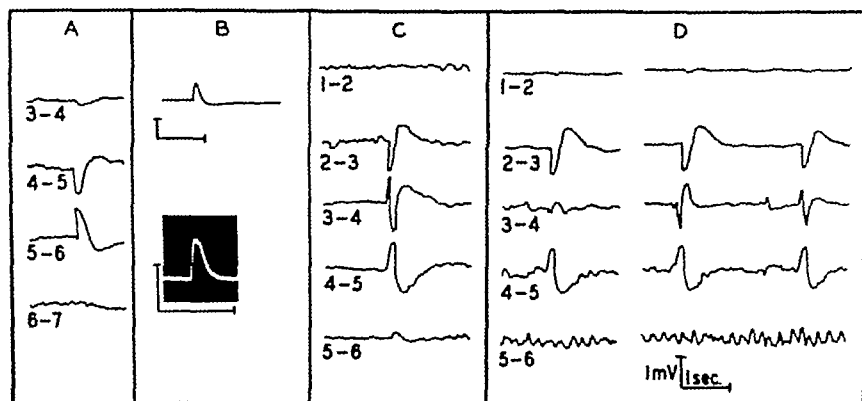


FIG. 12 Slow negative waves. Upward excursions denote negativity of the more frontal electrode (lower number) with respect to the more occipital electrode (higher number). Depression spreading from the frontal to the occipital regions. In A, C and D the electrodes were arranged in a row in the fronto-occipital direction at distances of 2 mm. A: Since leads 3-4 and 6-7 do not show any deflections, the wave recorded by 4-5 and 5-6 corresponds to a local change at 5. B: Upper record: ink-writing oscillograph with large coupling condensers in the amplifiers (calibrations—voltage 3 mV, time 1 sec). Lower record: cathode ray oscillograph and direct-coupled amplifiers (calibrations—voltage 2 mV, time 1 sec). C: Asynchronism of slow waves at two neighboring electrodes. Negative waves occurred at electrodes 3 and 4 (leads 2-3 and 4-5 are unaffected). The asynchronism is shown by the diphasic record 3-4. The wave at 3 preceded slightly that at 4. D: Changes in the starting point of negative waves at neighboring electrodes (3 and 4). The first discharge was synchronous, as shown by the absence of any significant deflection in record 3-4. The break in the records corresponds to a 4 sec. interval. The last two waves are asynchronous—first electrode 4 leads (the diphasic wave in 3-4 begins downwards), then electrode 3 leads (the diphasic wave 3-4 starts upwards).

an electrogram with no distortion, taken with a direct-coupled amplifier and a cathode-ray oscillograph is included for comparison. The records show the typical quick development of the negativity, and its slower subsidence. The slow negative waves appeared in any region of the cortex as soon as the spontaneous activity was depressed there. They occurred in variable number, one, a few separated by long intervals, or a rapid series lasting for 20 or more seconds. They then disappeared from this region. As the peak of the wave of depression successively reached different parts, the waves appeared successively in these different regions. Figure 13 shows that, as depression spread from electrode 1 to electrode 7, the slow excursions were recorded at different times at each of the electrodes. Figure 14 illustrates a typical group of slow negative waves in one region.

the cortex in the direction of the spread of the depression, the successive initiating foci inside this zone were irregularly distributed

As depression spread over the cortex, negative slow waves, if they did appear, they did not necessarily appear successively in all regions involved. They commonly took place only in some regions, or they might also be present in two distant regions, while the remainder of the cortex showed only

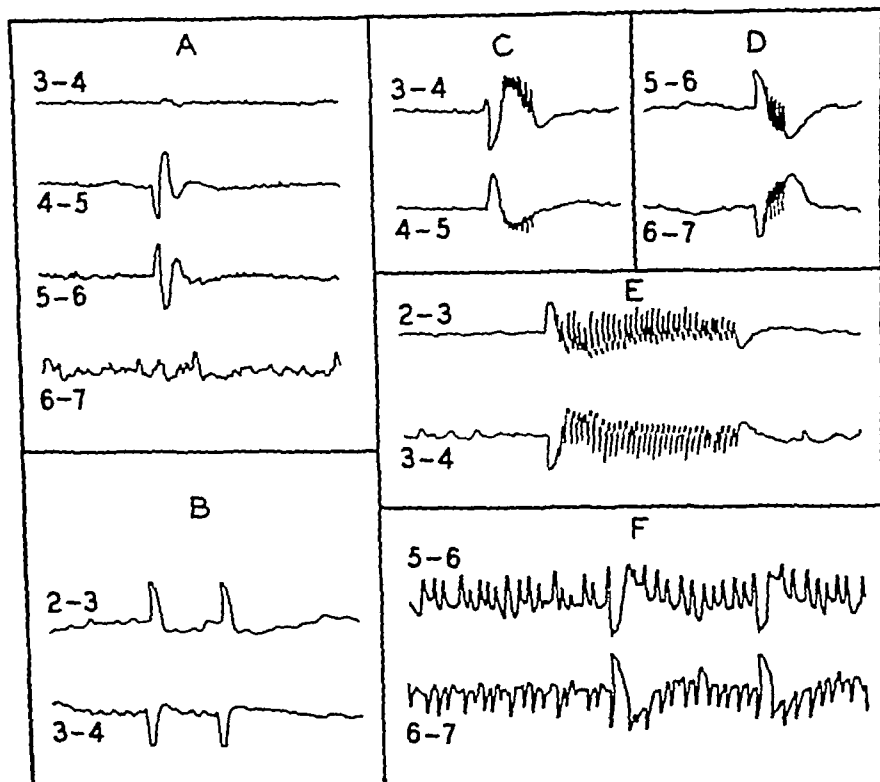


FIG. 15 A Diphasic wave at electrode 5 B Positive waves at electrode 3 C Burst of spikes following a slow negative wave D Burst of spikes following a slow positive wave E Long series of spikes, following a positive wave F More or less continuous series of spikes, and negative slow waves

depression. This indicates that the initiation of this activity depends on local conditions.

Single shocks applied to the pial surface of a region during the time in which negative slow waves were occurring there, could elicit responses entirely similar to these waves. Shortly after a spontaneous wave, however, single shocks always failed to elicit another one. If repeated at a frequency not much different from that at which the waves were recurring, the single shocks could control their rate of appearance, and also apparently prolong

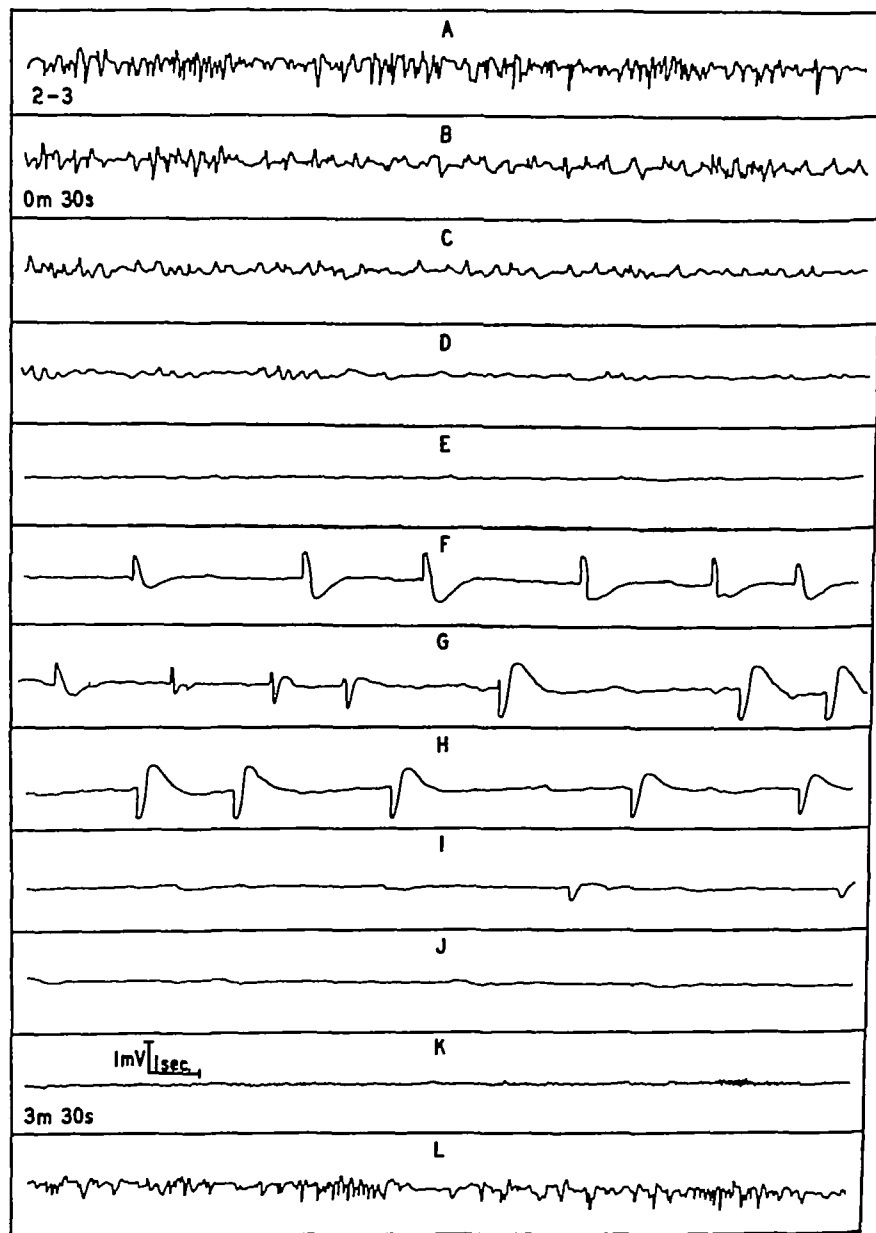


FIG. 14 A typical group of slow waves in one region. Electrodes arranged as in Fig. 3 and 13. A Spontaneous activity. B to J A continuous record, starting 30 sec. after stimulation, and showing the slow negative waves. K Beginning of recovery (in the interval, about one-half min., between J and K the record was in as J). L Recovery, 15 min. later. Compare strips F and H of this figure with D and E of Fig. 13.

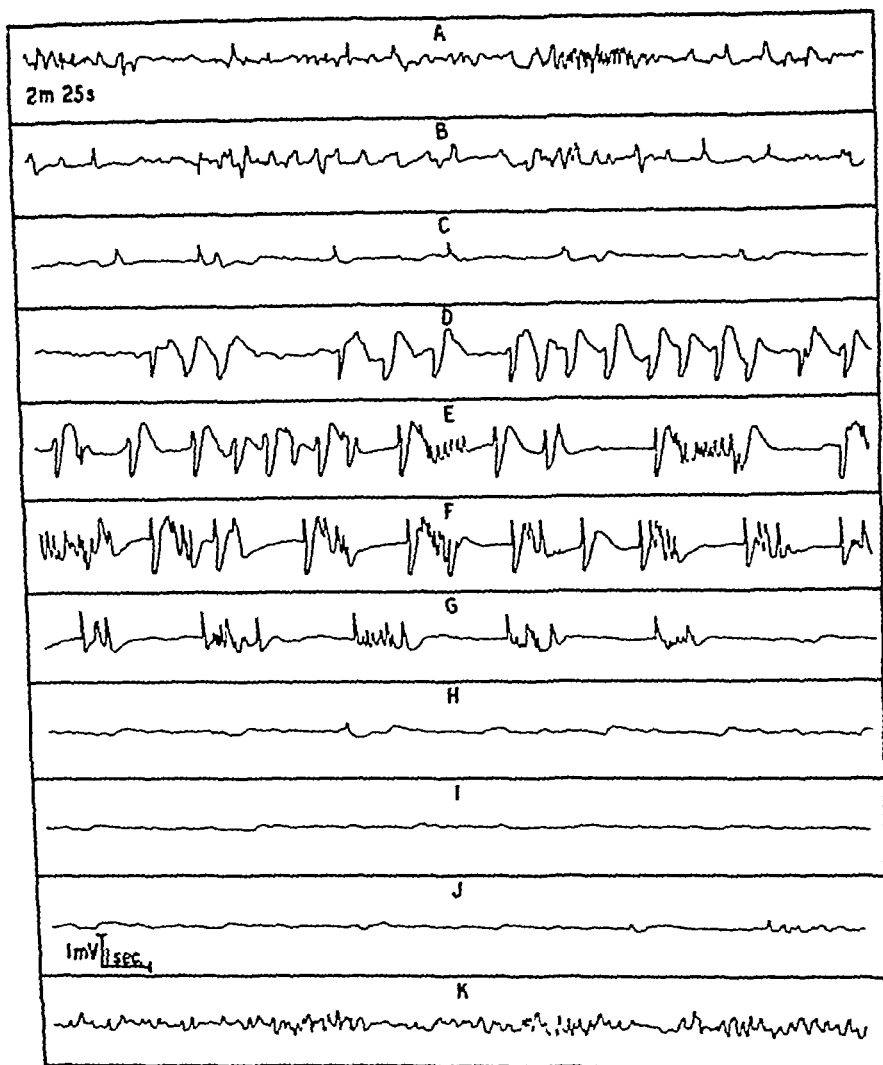


FIG 16 Intense activity occurring when the spreading depression reaches a given region A to J Continuous record, beginning 2 min 25 sec after a distant stimulation that elicited a depression K Recovery, 5 min later

activity, in one animal, at a given time, were similar for a number of successive stimulations. Variability was found from animal to animal. Striking variability also took place, as a rule, in the course of a given experiment—more activity was seen during the periods of depression when the hemispheres had been exposed for several hours and stimulated repeatedly.

C Activity similar to "self-sustained" (tonic-clonic) responses of experimental epilepsy. When the activity attending depression was intense it followed, in many cases, a course quite similar to that of the discharges that

this type of activity Sensory stimulation could likewise "drive" (39) the slow waves in the corresponding receiving areas

No point was found on the pial surface that was positive with respect to a distant electrode while a negative wave was recorded from a neighboring point (1) As long as the negative phenomenon did not spread from one electrode to another, interelectrode distance did not affect the features of the waves in the records These facts may suggest that the active elements are probably oriented perpendicularly to the surface

In some cases, diphasic or more complex slow waves were recorded under conditions which showed that the pattern was not caused by activity spreading from one electrode to another Slow positive potential waves were also recorded in many experiments (Fig 15A and B) As a rule, however, these waves appeared only when the activity occurring during the depression of the spontaneous pattern was more intense, *i e*, when it consisted of a more or less continuous, irregular succession of large slow deflections, or when fast potential waves were also present

B *Fast potential waves* The slow potential waves described in the preceding section were, in many cases, followed immediately by rapid, spike-like potential changes These spikes were usually "positive" They occurred in bursts, as shown in Fig 15C, D and E On other occasions, spikes appeared in the intervals between the slow waves in a somewhat continuous succession (Fig 15F) In many instances, finally, only a series of spikes, and no slow waves, was seen during the depression of the spontaneous activity

When, in one region of the cortex, slow waves and spikes occurred in rapid succession, a variety of complex patterns resulted (Fig 16 and 17) It was then sometimes really impossible to distinguish slow waves and spikes, waves of many different shapes, amplitudes and durations were recorded In these cases, especially because the recovery of the spontaneous activity was usually prompt, it would be inappropriate to speak of depression in the corresponding region In other regions, however, reached by the same wave of depression, before or after the particular region where intense activity occurred, only reduction of the spontaneous activity, or reduction and a few slow waves, was seen Intense activity was very rarely present at the frontal regions, it was more frequently seen in the posterior half of the hemisphere In a few instances activity was so marked in most regions, and the spontaneous pattern was recovered so quickly, that rather than a depression what was seen was a traveling band of modified activity the regions in front of this band had their "normal" spontaneous background, the regions immediately behind it were somewhat depressed, those further behind showed a well-recovered normal pattern The rate of travel of this band was about the same as when only depression of the spontaneous activity occurred

If the stimuli used were well below threshold for the production of an electrical after-discharge, the occurrence of slow waves or other activity in a given region was the same regardless of where the wave of depression was started The regional effects of a spreading depression of the spontaneous

also indicated by the following observations. In some animals an area was found which exhibited self-sustained discharges when a distant point was stimulated. Then that area was stimulated locally with sufficiently strong shocks. A first self-sustained response was followed after an interval of several seconds by a second similar discharge. This second period of activity coincided with the initiation of a typical spreading wave of depression. Thus

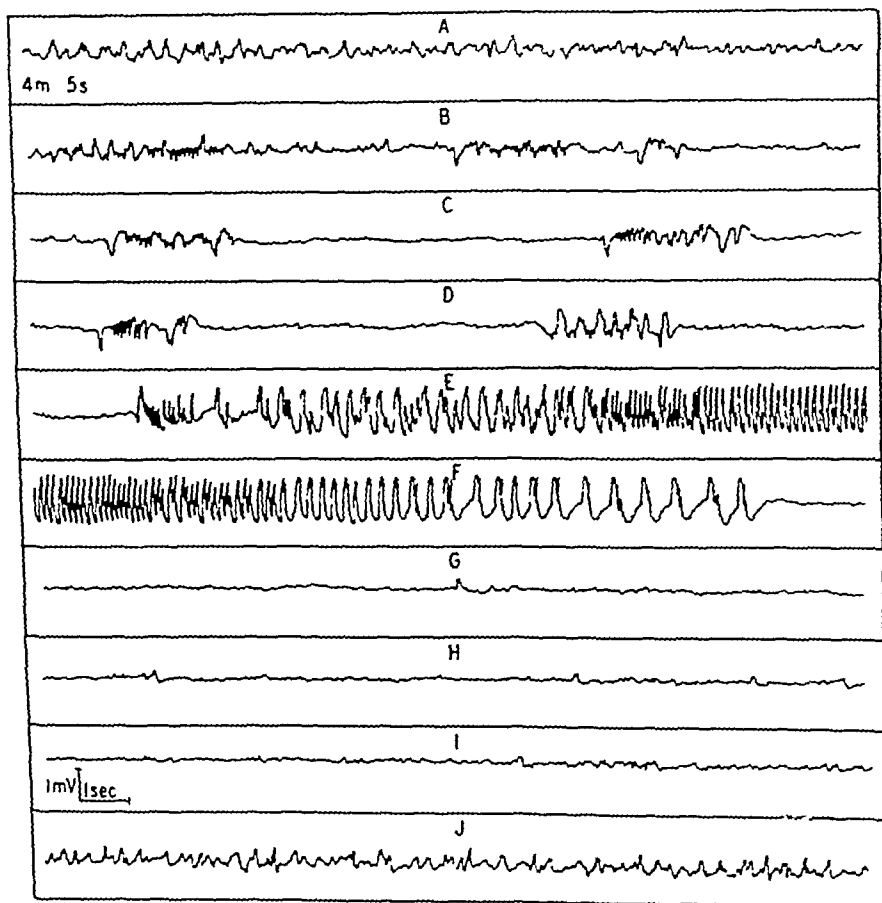


FIG. 18. Electrodes, etc. as in Fig. 16. The activity here is quite similar to a typical tonic-clonic response. A to I. Continuous record. J. Recovery.

it appears that the first period of activity was a direct response, but the second was an indirect effect, consequent upon the process responsible for the depression (Fig. 19).

OTHER ANESTHETICS, EXPERIMENTS ON PIGEONS AND CATS

With rabbits under nembutal the results were quite similar to those obtained with dial. The same was true when chloralose was used. On the other

follow a period of a few seconds of strong stimulation of the cerebral cortex Figure 18 illustrates this similarity (38) This is a typical "tonic-clonic" discharge, but it is important to emphasize that the stimulated region and the intervening areas did not share in the self-sustained activity recorded In the

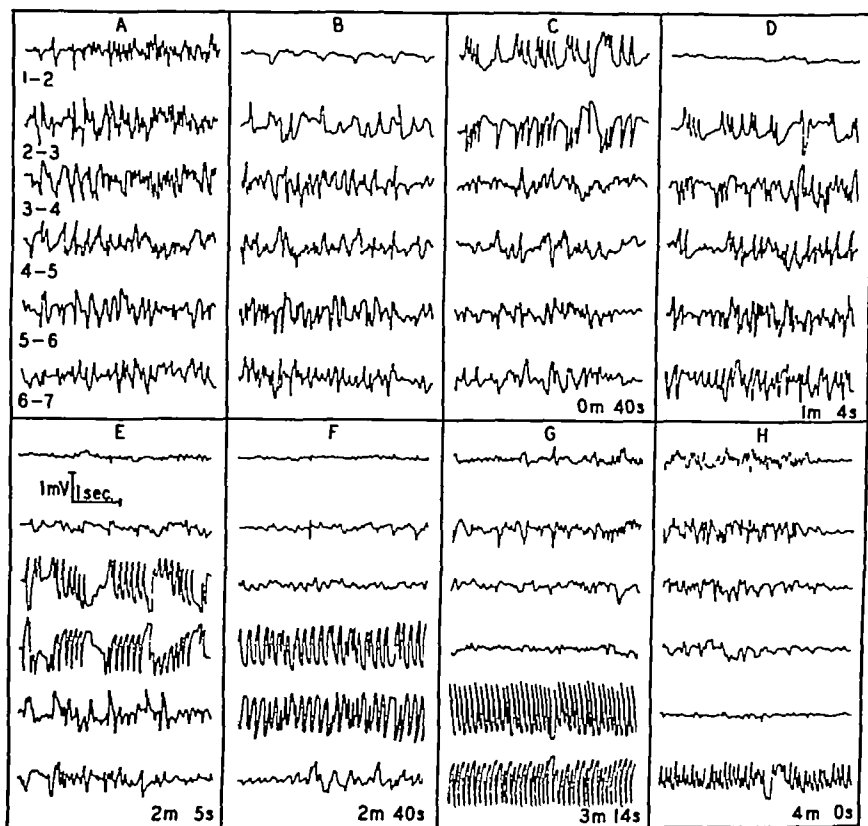


FIG 17 Very intense activity associated with depression, occurring in all regions tested Electrodes arranged as in Fig 3 A Before stimulation B Immediately after stimulation The discharges in electrodes 5 and 6 resemble typical tonic-clonic sequences

stimulated region and the intervening areas there was only depression of the spontaneous activity (and some isolated slow waves) The self-sustained discharge might appear only three or more minutes after stimulation if the stimuli were applied to a distant region These discharges were usually seen in the posterior half of the hemisphere They were, like the slow waves, limited at a particular time to a certain region of the cortex, and if they moved to another region, the rate of travel was the same as that of the slow waves (*i e*, the same as that of the depression of the spontaneous activity)

That the self-sustained activity was not a direct consequence of the stimuli, but an indirect effect caused by the mechanism of depression, was

The term "inhibition" has been used to cover many, probably heterogeneous, central phenomena. It has also acquired several specific interpretative connotations. For these reasons this term was avoided, and the purely descriptive word "depression" was selected.

The close relations between the spreading depression and the discharges of experimental epilepsy may first be stressed. On page 377 it was pointed out that typical discharges of experimental epilepsy may occur in a cortical region when it is reached by a spreading wave of depression, and that in certain cases such discharges take place successively in different regions as the wave of depression reaches them. Indeed, as a rule, whatever the type of activity that appears during depression, some features or properties of the tonic-clonic discharges (38) may be recognized. It may be said that the electrical events are always of a "convulsive" character.

The most frequent and simplest type of activity consists (page 376) of large, slow potential waves. When these waves recur in one region, this recurrence exhibits features similar to those of the repetitive clonic bursts of the cortical responses of experimental epilepsy. During the clonic bursts (1, 38), the regions inside the active area are coupled, i.e., activity in one is promptly followed by activity in the others (synchronism of the clonic bursts), but there is not a systematic pacemaker—the bursts originate at different, irregularly distributed points. A similar situation may be recognized inside the depressed cortex in which slow waves are recurring. Here also, activity starts at different points and is immediately followed by discharge in others inside the region involved. The conditions for driving the slow waves with single electrical shocks applied to the region follow the same general rules which govern the driving of the clonic bursts (39). The slow waves, as well as the spikes that may accompany them, show at times a striking resemblance to isolated components of the clonic phase of the self-sustained responses as described by Rosenblueth and Cannon (38). The slow waves are similar to their component IV, and the spikes to component III—the two components usually associated in the clonic bursts. Therefore, it seems reasonable to infer that the different types of activity which may appear during depression are closely related to each other and to the tonic-clonic responses. The differences seen in various instances are mainly quantitative.

There are two fundamental facts about the cortical discharges of experimental epilepsy which should be mentioned at this point. First, rapid excision of the stimulated area does not stop the response and does not prevent further spread (see Munk, in Wernicke [40], 13, 25, with regard to motor convulsions, and 38, with regard to the electrical phenomena of the cortex). This fact indicates that the area stimulated starts, but does not remain in control of the response and its spread. In the response studied here the stimulated area may return to its normal spontaneous activity, after a period of depression, at a time when depression is starting at some distant region. This indicates that here also the stimulated region initiates the response, but

hand, in animals under ether, responses of the type described were obtained only when the anesthesia was very light, inadequate for prolonged observations

In pigeons (nembutal) a spreading depression of the spontaneous electrical activity was easily obtained. The process had the same general features as described for the rabbit. The electrodes were placed on the dorso-lateral wall of the hemisphere. A survey of their position with relation to the

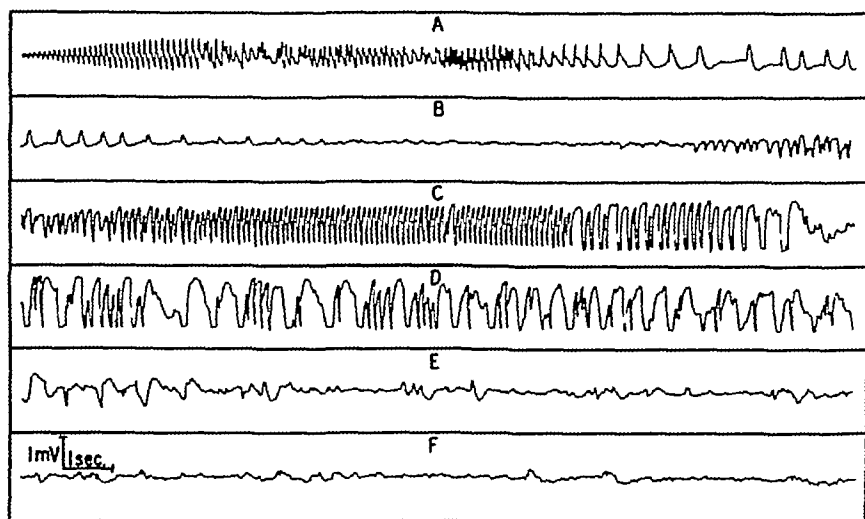


FIG 19 Immediate and late tonic-clonic responses to local strong stimulation. The stimulating electrodes were about 2 mm away from the recording leads. A to F: Continuous record, starting immediately after the stimulus. The second period of activity coincided with the initiation of a typical spreading wave of depression.

various cortical or corticoid areas that have been recognized in the avian brain (29) has not yet been completed.

A spreading depression of the electrical activity was also observed in the few experiments made on cats. As in the rabbit, large slow potential waves appeared in many instances during the depression. In these few experiments stimulating and lead-off electrodes were placed along the suprasylvian gyrus. In almost all the animals the brainstem had been transected under ether and the anesthetic then discontinued.

DISCUSSION

The data are not yet sufficient for an adequate discussion of the subject. Some surmises, concerning the changes in cortical function which might lead to the depression and the factors which might intervene for its spread, may be considered. These surmises are of value in that they suggest future interesting experiments.

prolonged synchronous clonic activity over a wide extent of cortex, is not obtained. For this reason the comparison of the rates of spread of tonic-clonic activity and of depression could not be carried out.

It is interesting to note that the results in pigeons, as well as those in cats (when the brainstem had been transected), were essentially similar to those obtained in rabbits. It is likely, therefore, that species differences may not be prominent and that the anesthetic may not markedly influence the phenomenon.

The fact that the depression may not spread beyond a narrow band of coagulated tissue, and also that it does not spread to the retrosplenial area, seem to point to a neuron-to-neuron mechanism for the spread. It is possible that some elements are stimulated by the electric shocks, and that the activity of these elements results in inhibition of the activity of many other neurons. A wave of marked vasodilatation and increased blood flow travels over the cortex simultaneously with the wave of depression (32). The most likely mechanism by which the response of the pial vessels might be produced seems to be a release into the interstitial fluids of some chemical vasodilator. Whatever the mechanism of this vasodilatation, it is to be expected that the marked increase in blood flow will in turn influence the activity of the cortical neurons in the region concerned. That is, the increased flow might, for instance, lower the carbon dioxide tension and, therefore, alter the functional activity of the nervous elements (28, 33). The time course of the depression of the spontaneous electrical activity in a given region is therefore probably affected by this mechanism. Also, it is possible that the chemical vasodilator itself, or some other chemical released in the tissue fluids, alters the activity of the nervous elements and so conditions directly the time course of the depression in any given region. A lack of mutual excitatory facilitating interaction among a group of elements, if some of them were inactivated, might also have a part in the development of the depression.

If the initiation and spread of the depression are due to the discharges of certain elements, then these discharges should have electrical signs. Hence it would be expected that some specific change in the electrical activity should be manifest at the front of the wave of depression. There was no evidence of such change in the records. It is necessary to assume therefore, that the active elements are distributed irregularly in the cortex, with no definite spatial orientation in relation to the electrodes, so that their discharges do not set up a recordable potential difference between the electrodes, i.e., the units of electrical activity may largely cancel each other. The complexity of the cortical structure permits such an assumption. The spread of depression may then be accounted for by synaptic activation of similar elements in adjacent regions. This interpretation implies that active depressing elements should be present in all cortical regions to which depression spreads. The fact that a wave of depression may be started at any of those regions supports the interpretation. The slow propagation, a difficult point to explain on the basis of synaptic activation of adjacent elements, might be due to the need

that the progress of the spreading depression is not controlled by an enduring influence from this region

The second noteworthy fact about the tonic-clonic responses is that the course of the activity is similar in any of the several regions to which it may spread, including the region stimulated. With regard to motor convulsions, François-Franck and Pitres (25) noted that, "dans l'ensemble de l'attaque, chaque membre . fait independamment des autres membres, une phase tonique et une phase clonique." Rosenblueth and Cannon (38) found that the electrical discharges in any cortical region start with the first, rapid component of the responses, and then pass into clonic bursts coupled with those in the rest of the active cortex. This fact indicates that the response starts anew in any cortical region, much as in the region stimulated. These authors stated that, "it is not necessary to assume that other elements than those which yield component I are involved in the spread of the responses, since activity of any area always starts with this component." Similarly, the time course of the spreading depression is the same in all regions involved, *i e*, the response again starts anew in distant places in much the same manner as in the area stimulated.

Rosenblueth and Cannon (38) observed that in any region along the spread of the cortical epileptic discharges, "the responses are usually preceded and attended by inhibition of the spontaneous activity." This observation supports the close relationship between the spread of the depression and that of the tonic-clonic activity. In the monkeys studied by Rosenblueth *et al* (38,39) the spread of the cortical tonic-clonic electrical activity, induced by electrical stimulation, took place more readily in the backward than in the forward direction in one hemisphere. Similarly, in the rabbit's brain, spread of the depression of the spontaneous electrical activity was more readily obtained when the frontal regions were stimulated than when the stimuli were applied to regions near the occipital pole.

From all these facts it is inferred that the mechanisms of spread of the waves of depression and of tonic-clonic activity involve the same cortical elements. It is also inferred that depression and tonic-clonic activity are closely related, *i e*, the elements which cause depression and its spread may in appropriate conditions activate the elements involved in the tonic-clonic responses of experimental epilepsy. A corollary of these inferences is that *the spread of tonic-clonic activity may be produced by the elements which cause the initial depression, rather than by those responsible for component I*.

A closer consideration of the inferred correlation of the two responses cannot be made at present. Thus it would be important to determine if the rate of spread of depression and that of tonic-clonic activity are the same in a given animal, under comparable conditions. Spread of tonic-clonic cortical activity has been studied in monkeys (23, 38). Whether in this species a spreading depression can be produced is still to be determined. In narcotized rabbits, typical gradual spread of tonic-clonic cortical activity out from a small area strongly stimulated for a few seconds, and resulting finally in a

activity in cortical neurons, but that it was due to potential gradients in afferent fibers from the thalamus. One fact supplying evidence of this origin of the positive waves was that similar waves could be recorded from the exposed white matter after removal of the cortex. Curtis (16) interpreted the early "positive" wave of the responses evoked in the opposite hemisphere by single shocks applied to the pial surface, as due to summation of axon spikes of the callosal fibers. As opposed to the view that these potentials are originated entirely in the afferent axons, a view shared also by Marshall, Woolsey, and Bard (34), more recently Dempsey and Morison (17) obtained evidence that cortical elements contribute to the initial positive wave of the sensory responses. The depression of these responses and of the responses to single shocks applied to the contralateral hemisphere is difficult to reconcile with the view that the initial positive wave is the manifestation of potentials in the corticopetal fibers (thalamic or callosal). It is quite unlikely that axon potentials (in the paths approaching the cortex) could be depressed or inhibited. The depression seems to indicate that the activity revealed by these positive waves corresponds to some cortical neurons. If the arrival of corticopetal impulses should record, with electrodes on the pial surface, as a positive potential this potential would be much briefer and smaller than the initial positive component of the typical records.

A second comment suggested by the present data concerning the interpretation of electrocorticograms is the following. Adrian and Matthews (3) concluded that the slow potential waves recorded from the cortex are summated effects built up from an asynchronous series of brief pulsations in nerve cells. One of the arguments they presented for the composite nature of slow waves was that the origin of those waves could not be localized. The records presented in Fig. 14 show that it is possible to localize the origin of the specific slow negative waves that may appear as depression reaches a cortical region. It is likely therefore that these waves are not composed from brief asynchronous discharges in elements distributed over a wide area. These waves might represent prolonged potential changes in relatively few elements or else they might have an origin of an entirely different nature.

There is obviously a similarity between the response described in the present study and the phenomenon of "suppression" of the electrical activity, described first by Dusser de Barenne and colleagues (4, 19-22, 26, 27). There are, however, many differences between the two responses. Suppression of the electrical activity in the experiments of these authors is brought about by the influence of specific cortical areas on the activity of the nucleus caudatus, which in turn by way of the thalamus influences the activity of the cortex. In the present experiments the evidence indicates that the characteristic spread is a cortico-cortical process. In addition, since the gradual spread progresses uniformly, since the same events repeat in each cortical region, and since there is no correlation in time of the events at distant points with those at the stimulated area, it is clear that the mechanism responsible for depression is widespread over the cortex rather than the attribute of spe-

of summation of excitation over a long period of time before new elements are activated. Since the spread within one hemisphere is to the regions in the vicinity of a given depressed area, it should take place mainly through short neuronal connections. On the other hand, the fact that the effect can be elicited in the contralateral hemisphere seems to indicate that long pathways may be involved.

During the development of depression in a region not only was the spontaneous activity decreased, activity which in itself is to be regarded as a sum of components (17, 36) but in addition many different types of cortical responses were eliminated. This may be considered evidence that there exists in the cortex a mechanism for a diffuse inhibitory influence. Changes in the physico-chemical composition and equilibria in a given depressed region, as seemingly evidenced by the changes in the pial vessels and as probably brought forth by the marked and sudden increase in blood flow, may have an important part in the realization of this diffuse cortical inhibition. They might account for the fact that a second spreading depression cannot be elicited from the same region until the recovery of the electrical activity is well advanced there.

The depression illustrated in Fig. 11 II may be singled out, in that decrease of response appeared when a wave of depression reached not the responding but the stimulated region. It is possible that depression may decrease the electrical excitability of cortical elements. On the other hand, it is unlikely that the excitability of the callosal fibers should be modified. Probable explanations are that the stimuli act on the cell bodies of the projecting neurones, but that then threshold had been elevated, or else that the stimuli activate directly other elements than the projecting neurones.

Several of the ipsilateral responses that can be obtained by direct cortical stimulation with single shocks were studied by Adrian (1) and by Rosenblueth and Cannon (38). These responses exhibit several components with different features. This complexity suggests that several different sets of elements may be involved. A detailed study of the depression of these responses, with different positions of the stimulating and recording electrodes in relation to the direction of spread of depression, so that either the stimulating or the recording pair is reached by the depression, one slightly before the other, should contribute significantly to their understanding, and to that of the mechanism of depression. The observations made here (page 384) are considered only an introductory step in this study.

The depression of tonic-clonic responses is difficult to explain. Spreading depression may cause the appearance of tonic-clonic activity. The two phenomena are closely related, yet in certain conditions depression opposes the initiation of a tonic-clonic response.

Some of the results mentioned in the preceding pages may have a bearing on the interpretation of the potential waves recorded from the surface of the hemisphere. Rosenblueth (24) and Adrian (2) suggested that the initial sensory responses did not result from

clusively cortical, *i e*, they do not require a contribution from sub-cortical centers. Their development and characteristics are not determined by the stimuli, but depend on the local characteristics and conditions of the affected regions.

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cific areas A further difference is that specific electrical activity often occurs during depression No such activity has been reported in the suppressed cortex A closer comparison of the two responses awaits further studies

I wish to express my cordial gratitude to Dr Hallowell Davis This study was developed under his continuous, valuable and friendly supervision My daily contacts with Dr Arturo Rosenblueth, who followed the experiments closely, were a decisive factor in the completion of this work In all its phases, his help was beyond estimation

SUMMARY

In rabbits, under dial narcosis, weak faradic or mechanical stimulation of the exposed cerebral cortex elicits a characteristic response designated "spreading depression"

Shortly after the stimulation the spontaneous electrical activity decreases markedly at the stimulated region (Fig 1) The depression then slowly spreads in all directions, successively affecting adjacent areas Within 3 to 6 minutes it involves all of the dorsolateral cortex, except the area retrosplenialis granularis dorsalis (Rsg β , of Rose) (Fig 2-5) The depression can be initiated in any of the regions involved in the spread, but is usually more readily obtained from the frontal than from the occipital areas

Recovery of the initial pattern of spontaneous activity is slow, it requires, as a rule, 5 to 10 minutes at each region The spontaneous activity at the stimulated region is often well recovered at a time when the depression is just starting in distant parts If only weak stimuli are used, depression at any region runs the same course regardless of what region is stimulated

Only with supraminimal stimulation does the depression spread to the opposite hemisphere, then appearing first in the region symmetrical to the point of stimulation and thence spreading as in the stimulated hemisphere

The spreading depression affects not only the spontaneous electrical activity, but also several cortical responses, *i e*, responses to touch, to electrical stimulation of afferent nerves, to illumination of the retina, to ipsilateral or contralateral cortical electrical stimulation, and to local application of strychnine or of eserine plus acetylcholine (Fig 6-11) Single shocks applied to a depressed region fail to elicit typical responses in the opposite nondepressed hemisphere

Specific activity, different from the spontaneous, often develops during the period of depression of a region The most common type of this activity is composed of large, slow, localized potential waves, during which one electrode becomes negative with respect to others 1 to 3 mm distant (Fig 12, 13, 14) Fast components may also appear, and the activity when intense closely resembles the "seizure pattern" of experimental epilepsy (Fig 15-19)

The depression and "tonic-clonic" activity of experimental cortical epilepsy seem to be closely related phenomena The spread of tonic-clonic responses is probably mediated by the same cortical elements which are involved in the spread of depression The two processes are mainly or ex-

PIAL CIRCULATION AND SPREADING DEPRESSION OF ACTIVITY IN THE CEREBRAL CORTEX

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THE SPREADING depression of activity, a response elicited by electrical or mechanical stimulation of the cerebral cortex, has been described by Leão (8)

Briefly, this response consists of a marked, enduring reduction of the electrical activity, a reduction which appears first at the region that has been stimulated, and spreads out from there in all directions involving successively more and more distant parts of the cerebral cortex. The rate of spread is slow. In rabbits, under dial narcosis, a response started near the frontal pole may take more than 5 minutes to reach the occipital pole. Recovery of the initial pattern of spontaneous electrical activity requires 5 to 10 minutes, or even more, at each region.

The present paper describes changes that have been observed to occur in the pial circulation simultaneously with the depression of the cortical electrical activity and its spread.

METHODS

Rabbits, under dial narcosis (0.55 to 0.75 cc per kg, administered by intraperitoneal injection) were used in all experiments. One or both cerebral hemispheres were widely exposed. The pial vessels were observed with a compound microscope. The beam of light directed to the surface of the hemisphere was filtered through an ammoniacal copper sulphate solution, in order to remove heat rays and provide better color contrasts.

The stimulating electrodes—fine silver wires, with a small bead at the tip—were applied to the pial surface. The interelectrode distance was about 1.5 mm. The stimuli were "tetanizing" shocks from a Harvard induction coil, delivered for a period of 3 to 5 seconds. The stimulation was always below threshold for the production of any immediate cortical electrical "after-discharge." Mechanical stimulation was obtained by means of a few light touches with a small glass rod. These stimuli caused only a slight compression of the tissues, without any visible structural damage. A six-channel Grass ink-writing oscillograph was used for the study of the cortical electrical activity (8).

RESULTS

The arteries and veins of the pia are readily identifiable. Besides distinctive features in their course and manner of branching, their difference in color is very striking—the arteries are a bright scarlet, the veins a purplish red. The blood flow in the arteries is ordinarily too rapid to be followed, but in the veins it is clearly visible. The entire picture of the pial circulation is markedly changed when, following stimulation, depression of activity spreads over the cortex.

1. At the stimulated region, as the electrical activity becomes progressively more and more depressed, a very conspicuous dilatation of the arteries occurs. In the veins, the rate of flow is strikingly increased, and these vessels

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If, following supraminimal stimulation, a depression of activity appears in the opposite hemisphere, then vasodilatation and increased blood flow also occur in that hemisphere. The changes in the pial circulation are first seen at the region symmetrical to the one stimulated, and spread out from there to the rest of the hemisphere in exactly the same manner as described for the stimulated side. The latency for the appearance of the depression of activity, and the concomitant changes in the pial circulation, at the symmetrical region is longer than at the stimulated region, so that the spreads in the two hemispheres are not coincident. The response on the opposite side, starting a little later, as a rule covered at any given time a smaller area than that involved on the stimulated side. The changes in the pial circulation are similar in a given region, whether the stimuli have been applied near or far, and regardless of the region stimulated.

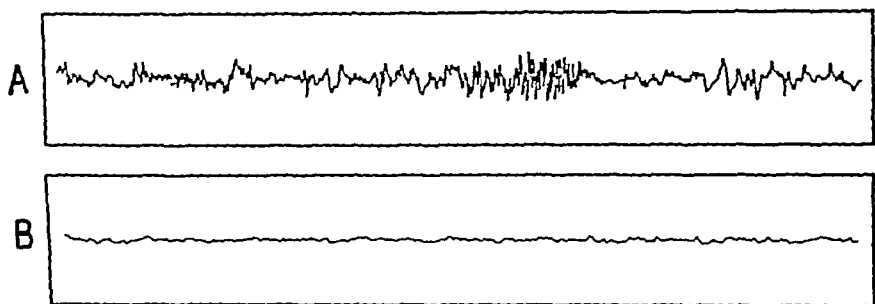


Fig. 2. Electrograms taken from electrodes in the immediate neighborhood of the area shown in Fig. 1. A: Control, before stimulation. B: The depression of electrical activity, taken during the exposure of Fig. 1B.

3. Specific electrical activity, clearly different from the "spontaneous," often appears during the depression of cortical activity. This activity is of a "convulsive" character, and when intense closely resembles the "seizure pattern" of experimental epilepsy, (8). The response of the pial vessels is similar whether pure depression of the spontaneous electrical activity takes place or whether specific increased activity of any intensity develops during the depression.

4. In many experiments, the systemic arterial pressure was recorded while observations were made on the pial circulation and on the electrical activity of the cortex. One carotid was cannulated and connected to a membrane manometer. No change of the blood pressure took place while the cortex was stimulated and the wave of vasodilatation and increased blood flow spread over the hemispheres.

COMMENT

The factors regulating the blood flow in the brain have been reviewed recently by Forbes (3), who tabulated the extracerebral and the cerebral factors. In the phenomenon observed in the present experiments, the changes in

promptly become as scarlet as the arteries. Many small vessels, unnoticeable before, become clearly outlined in the field.

From the stimulated region similar changes spread out slowly in all directions. This spread is strictly analogous to that of the depression of the electrical activity, and the two processes appear coincidentally at any given region. That is, the wave of vasodilatation and increased blood flow successively affects adjacent areas and within about 3 to 6 minutes involves all of the dorsolateral aspect of the cerebral hemisphere of the rabbit. As in the case of the depression of the cortical potentials, the only area not involved is a small region medial to the parasagittal sulcus. This area corresponds to the cytoarchitectonic area "retrosplenialis granularis dorsalis" (Rsg β) of Rose (11).

At any given region, maximal dilatation of the arteries is reached in about 0.5 to 1.5 minutes after it first becomes noticeable. The gradual subsidence



FIG. 1. A. Control before stimulation. The large vessel is an artery, of about 120μ diameter. B. The wave of depression has reached the region (see Fig. 2). The diameter of the artery is approximately doubled.

is somewhat more variable in time. As a rule, however, within 1.5 to 3 minutes the caliber of the arteries has returned to previous values. Arteries of all sizes are strongly dilated. The increases in diameter, as observed with the microscope, are of the order of 50 to 100 per cent (Fig. 1). Although precise measurements with an ocular micrometer are difficult to make, due to the pulsations of the exposed hemisphere (chiefly those caused by the respiration), the curve of Fig. 2 illustrates the general course of a representative observation.

Occasionally, the period of marked dilatation was followed, in some arteries, by a long period of a relatively much slighter reduction of caliber. The change in the pial circulation was in most cases easily perceived with the naked eye, as a widening area of a reddish color.

2. With minimal effective stimuli, the wave of vasodilatation, like the depression of activity, spreads only in the stimulated hemisphere. No change takes place in the pial circulation of the opposite hemisphere while the wave of vasodilatation is spreading over the stimulated side.

the spread of the response, the majority of the cells show a depressed activity, *i.e.*, they probably produce less CO_2 , so that a rise sufficient to cause the conspicuous dilatation observed is doubtful. As an additional argument it may be mentioned that in many experiments the small veins were carefully observed in order to find out whether they became darker before they turned as bright scarlet as the arteries. No such darkening was seen. Although the vessels of the brain are highly sensitive to CO_2 , this agent would act mainly on the fine, readily permeable vessels. A large concentration would be necessary to produce the marked dilatation of large arteries seen here. Such high concentration would presumably be associated with high O_2 consumption. Some darkening of the blood would, therefore, be expected to appear before the period of vasodilatation.

Some other metabolite, *e.g.*, a fixed acid, or a change in concentration of some inorganic ion, or some specific organic compound, might be the agent for the vascular reaction observed. Whatever the mechanism of the vasodilatation, it is to be expected that the marked increase in flow will in turn influence the activity of the cortical neurons in the region concerned (for instance, by producing acapnia, and so altering the functional activity of the cortex). The time course of the depression of activity in a given region is therefore probably influenced by the vascular reaction.

The cerebral blood flow during seizures induced by electrical stimulation has been studied by many investigators (4, 10, 2, 7) by means of thermocouples. Penfield (9) observed the pial arteries in epileptic patients during craniotomy. These authors report an increase of flow in the portion of the cerebral cortex involved in the convulsive discharge. Gibbs, Lennox and Gibbs (6) measured the jugular flow in epileptics and found also an increase accompanying seizures. In the present experiments, vasodilatation and increased flow always occurred in any cortical region when a wave of spreading depression of activity reached it. This increase occurred whether there was pure depression, or whether convulsive potentials appeared. Hence, the increased flow cannot be attributed to the increased neuronal activity of the discharge itself. The vascular reaction is correlated with the mechanism of depression and its spread, and in our experiments precedes the convulsive potentials that often occur in depressed areas.

The close relations between the spreading depression and the discharges of experimental epilepsy have been discussed by Leão (8).

I wish to express my gratitude to Dr. Hallowell Davis. This study, first reported in a thesis submitted October, 1943, in partial fulfillment of the requirements for the degree of Doctor of Philosophy, Harvard University, was carried out under his valuable and friendly supervision.

SUMMARY

In rabbits, under dial narcosis, a wave of marked dilatation of and increased blood flow in the pial vessels travels over the cerebral hemispheres concomitantly with the depression of electrical activity that is elicited by weak electrical or mechanical stimulation of the cerebral cortex. The two

caliber of the cerebral arteries seem to be active and correlated with local activity of neurons. That the vascular reaction is secondary to a local change in the activity of nervous elements is indicated by the cases in which depression of activity is elicited in the opposite hemisphere. Arterial vasodilatation and increased blood flow then appear at the region symmetrical to that stimulated. Only nervous pathways could establish this solidarity between symmetrical cortical regions in the two hemispheres. General factors are excluded by the fact that there were no changes in the systemic arterial pressure during the spread of the response.

It seems clear, therefore, that the change in the activity of the cortical neurons, when depression is starting in any given region, causes local vaso-

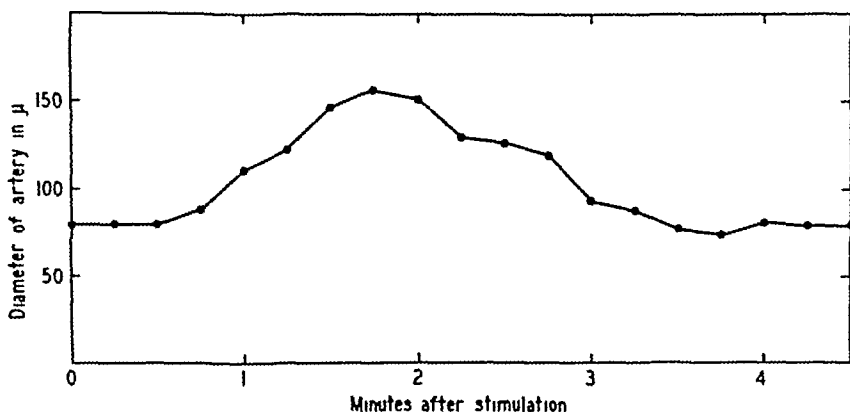


FIG 3 Graph showing the time course and magnitude of arterial vasodilatation attending a depression

dilatation and increased blood flow. An understanding of the inter-relations between the two phenomena awaits further investigation. The following surmises may be suggested. A chemical vasodilator agent might be released into the interstitial fluid; this is the most likely mechanism by which the response of the vessels might be produced. Another possibility would be a local, intrinsic system of vasomotor nerves. No evidence has ever been found of such a system, but the prompt and marked dilatation of large pial arteries that was observed in the present experiments might suggest this possibility.

Among the vasodilator substances, carbon dioxide is of special importance. That it is a powerful cerebral vasodilator has been established by several authors, and with different methods (5, 15, 1, 13). Regional increases in blood flow, correlated with local neuronal activity, and probably caused by increased cellular metabolism with a corresponding rise in CO_2 tension, have also been reported by several authors (14, 13, 12). Since the vasodilatation observed in the present experiments develops as the electrical signs of neuronal activity wane, a correlation with a rise in CO_2 tension is not probable. Although some cortical elements are probably active in bringing forth

INFLUENCE OF CONDITIONING NERVE STIMULI ON RELAYED VOLLEYS EVOKED FROM SPINAL CORD PERIODIC FACILITATION AND INHIBITION* †

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THE EFFECT of interfering stimuli on the time course of summation in the spinal cord was studied by Bremer (2) and by Eccles and Sherrington (4) with a reflex muscle contraction as index. Maxima of facilitation were found at different intervals and rather complicated curves for the time course of summation were obtained.

Today direct recording of the waves of potential, emanating from the ventral roots, would seem to offer new possibilities for the analysis of the time course of states of excitation and inhibition. The general principle of the method still remains the same. A conditioning stimulus sets up a state of excitation investigated by means of a second test stimulus. But in these new experiments the test stimulus is delivered by means of electrodes placed directly into the lateral region of the spinal cord, the conditioning stimulus being applied to the popliteal nerve of the same side. The reflex volleys are led off from the ipsilateral peroneal nerve. It proved possible to further the analysis by these means. Some basic findings, describing periodically recurring inhibitory and excitatory states will be presented in this paper.

METHOD

The experiments were carried out with decerebrated cats. A 4-stage push-pull directly coupled amplifier was used together with a double ray cathode ray oscillograph. To the one beam was led the effect from the peroneal nerve (see Fig. 1), the other beam recorded time

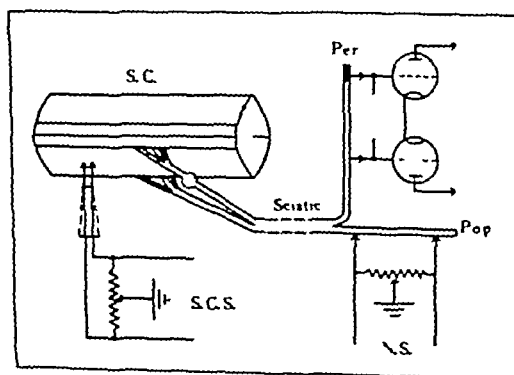


FIG. 1 Diagram illustrating experimental set up. S.C., spinal cord, Per, peroneal nerve (crushed at the end) with recording leads attached. Pop, popliteal nerve with stimulating electrodes (NS) for conditioning stimulus, SCS, stimulating electrodes for test stimulus to spinal cord.

* Aided by a grant from the Rockefeller Foundation for neurophysiological research.

† Of necessity it has not been possible to have proof for this paper read by the author and some of the references have been corrected and supplied in the editorial office in order to complete them.

processes appear coincidentally at any given region, and involve all of the dorsolateral aspect of the hemisphere, with the exception of a small region, medial to the parasagittal sulcus (area Rsg β , of Rose)

Arteries of all sizes are greatly dilated. The increases in diameter are of the order of 50 to 100 per cent. The flow in the veins is strikingly increased and these vessels promptly become as scarlet as the arteries.

The changes in the pial circulation are similar whether only depression of the spontaneous electrical activity takes place or whether "convulsive" activity, of any intensity, develops during the depression.

The vascular response is apparently secondary to a local change in the activity of nervous elements. Whatever the mechanism of the vasodilation, the marked increase in blood flow probably influences in turn the activity of the cortical neurons.

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The first volley with its total latent period of about 6 msec is followed after a latent period of 8–8.5 msec by a second volley and sometimes even by a third more or less well synchronized peak with a total latent period of 12–12.5 msec. Repeated stimulation at the frequencies used (10–20 per sec) seems to favour the appearance of synchronized volleys (7).

2 *Effect of conditioning stimulation on relayed waves elicited by test stimuli to spinal cord* The first volley elicited by the electrodes placed in the spinal cord (marked by a cross in Fig. 2a) is caused by direct stimulation of afferent or efferent fibres or of the ventral horn cells. It is followed by one or several, generally two, volleys with different latent periods. Their number and size depend upon the locus of the electrodes, stimulus frequency, excitatory background, etc. They have hitherto generally been regarded as waves relayed over one or several synapses but some of the results to be presented in this paper suggest that other explanations may have to be considered too. In Fig. 2a they are marked 1 and 2 and will be referred to as first and second relayed wave respectively. Facilitation of relayed waves has been described by Lloyd (7) and Renshaw (13). Bernhard and Granit (1) have shown that the frequency tolerance of such waves relates inversely to their central reflex times. In these experiments it has been difficult to elicit relayed waves in denervated animals, whereas they are commonly seen if the hind legs have been left innervated. For this reason innervated animals were used.

The experiments were generally begun by localizing a place in the spinal cord for which upon stimulation by the spinal cord electrodes, one or several well synchronized peaks followed. Such a place having been found, the conditioning stimulus to the popliteal nerve was started, and its effect upon relayed waves of different latent periods was analyzed for different time intervals between conditioning stimulus and test stimulus. The aim of the experiment was to determine whether the effect of the conditioning stimulus upon the relayed volleys, caused by the test stimulus, followed any general rules referable to latent periods and interval between the two interfering stimuli. In describing the results latent periods have been used instead of central reflex times in order to simplify interpretation of the figures. But in the final summary of the results all times have also been reduced to central reflex times.

A typical experiment will now be described with the aid of Fig. 2 in which the spinal cord electrodes are placed laterally 3–4 cm above the *crista iliaca*. In record *a* the direct wave follows the shock artifact after 1.7 msec which accordingly is the conduction time from the spinal horns to the recording electrodes on the peroneal nerve. It is succeeded by a first relayed volley with a total latent period of about 4.5 msec and a somewhat variable second relayed wave with a total latency of about 7.5 msec. Relayed waves as late as wave 2 of record *a* are seen only in good preparations and only if the electrodes have been well placed. In the limiting frequency diagram of Bernhard and Granit (1) the longest central reflex time for a homolateral relayed wave was 5.3 msec, corresponding to about 5.8 msec in record 2a.

in msec Both beams were shifted horizontally by a sweep circuit, the film being run vertically at slow speed The spinal cord was stimulated, as in the experiments by Bernhard and Granit (1), by needles, insulated except at the tip, and at a distance of 2 mm from each other (S C S in Fig 1) Stimulating electrodes to the popliteal nerve are marked N S in Fig 1

The stimulating circuits were controlled by two photocells, attached to movable radii fixed to a central common axis and activated by a rotating reflector in the same axis Any desirable stimulus interval could thus be obtained by shifting the photocells relative to each other along the periphery of the circle the centre of which was the rotating reflector A third photocell controlled the sweep circuit

RESULTS

1 *General description of reflex volley recorded in peroneal nerve upon stimulation of ipsilateral popliteal nerve* In good preparations stimulation of the conditioning popliteal nerve often elicits a set of successive reflex volleys at

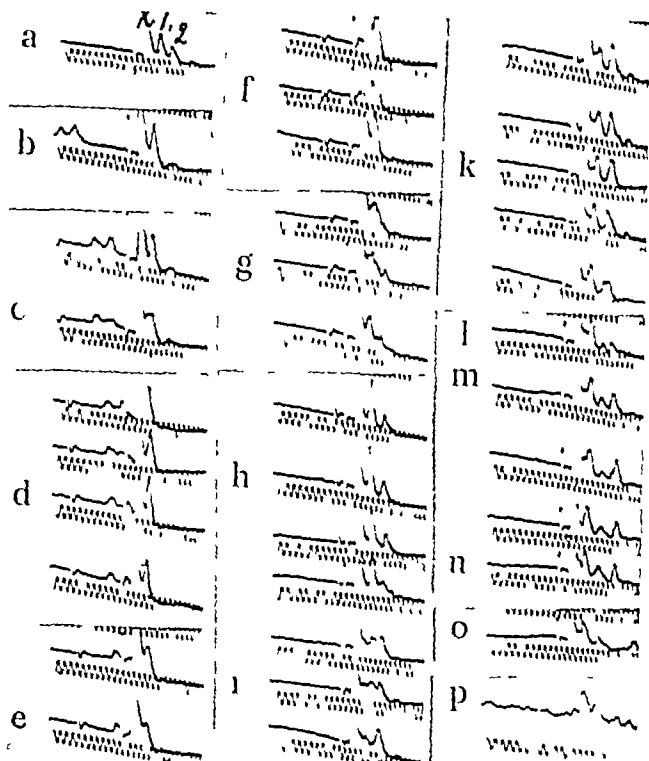


FIG 2 Responses recorded from the peroneal nerve upon stimulation of homolateral popliteal nerve (conditioning st) and spinal cord (test st) at different intervals between conditioning and test stimulus *a*, effect of test stimulus alone showing direct wave (τ) and two relayed waves (1 and 2) *b-n*, effect of shortening of interval between conditioning and test stimulus See text *p*, effect of conditioning stimulus alone at greater amplification

the recording electrodes on the peroneal nerve A picture of such a response is found in Fig 2 record *p* Its latent period is about 6 msec After subtracting from this value the peripheral conduction times on the afferent and efferent sides, together about 3 msec, there remains a central reflex time for the first volley of about 3 msec

cated in the following records *m-o*. But from the second relayed wave in record *l* is seen to escape a volley which from *m-o* diminishes, shifts to the right and drifts out of the picture in parallel with the shift of the artifact of the conditioning stimulus. This wave, to judge by its latency, is the first volley caused by the *conditioning* stimulus.

This experiment is a characteristic sample, chosen from a great number illustrating similar periodic facilitations and depressions. Eleven such experiments have been analyzed below in Table 1. In the diagram of Fig. 3 two similar experiments of this kind have been averaged. The intervals between the stimuli are found on the abscissa. Values to the left of zero refer to that part of the experiment (records *a-i* of Fig. 2) in which the conditioning stimulus precedes the test stimulus, at zero abscissa they coincide, and to the right of zero the conditioning stimulus succeeds the test stimulus. The ordinates illustrate the facilitations and depressions of a single relayed wave of long latent period, long enough to enable the conditioning stimulus to condition this wave even though its shock artifact just succeeds the shock artifact of the test stimulus. Normal amplitude is at ordinate 100. The figure illustrates the remarkable brevity of the facilitations and depressions.

The curve drawn in full represents the periodic fluctuations in the first relayed wave at different intervals between the stimuli caused by the interfering conditioning stimulus. There are three well marked facilitation maxima all of which push this wave above its normal level. They occur at intervals of respectively 8-9, 4-5, and 1-2 msec. Between these maxima are minima of inhibition pressing the amplitude below the normal level at 100. The behaviour of the *second* relayed wave with latent period 7.5 msec. is illustrated by the broken line. It can be seen that as long as the conditioning stimulus preceded the spinal cord stimulus by more than 4 msec. the second relayed wave was completely inhibited. Facilitations are found at intervals of 2 msec. *before* and 1-2 msec. *after* the test stimulus to the spinal cord.

There may be variations in number and size of the relayed waves connected with the general excitatory background and with the level of decerebration. But insofar as relayed waves are present they are always influenced by the conditioning stimulus at certain regular and well defined intervals differing little in time from experiment to experiment. The evidence for this statement will be taken up below in connection with Table 1. Even if certain relayed waves are lacking in the complex response, caused by the test stimulus alone, they may turn up at their expected facilitation maxima under the influence of the conditioning stimuli. Similarly, conditioning stimuli, which alone do not elicit any visible waves, facilitate and inhibit relayed waves, started by the test stimulus, at their proper time intervals.

3 *Short intervals, absence of direct wave*. The spinal cord electrodes may also be placed so that they cannot excite the dorsal or ventral root fibres directly (7, 8, 9). In such cases the response picked up in the peroneal nerve only consists of relayed volleys. An experiment of this type is illustrated in Fig. 4 in which the large wave following the second shock artifact in record

Depending upon the interval between conditioning and test stimulus relayed waves may be uninfluenced, facilitated or depressed. In record 2*b* the conditioning stimulus precedes the test stimulus by 16–17 msec so that its shock artifact falls outside the picture and its response just at the left end of the record, but in *c*, and still better in *d*, these artifacts can be seen, and the conditioning response (*cf* particularly *b* and *c*) consists of two small volleys with latent periods 6 and 8–8.5 msec. Greater amplification revealed a sporadic third volley after 12–12.5 msec.

Let us now examine the effect of the popliteal conditioning stimulus upon the complex response *a* of the spinal cord stimulus. In record *b* the first re-

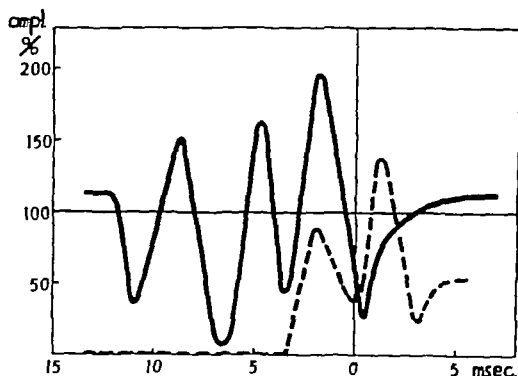


FIG. 3. Relative amplitude of relayed waves, caused by test volley to spinal cord, plotted against interval between conditioning and test stimulus. Curve drawn in full refers to wave of latent period 4.5 msec, curve in broken lines to one of latency 7.5 msec. To the left of zero abscissa conditioning stimulus precedes test stimulus, to the right vice versa. Amplitude in per cent of normal value at horizontal line 100.

elayed volley is greatly augmented, the second greatly depressed. Records *c*–*o* show what happens when the conditioning stimulus approaches the test stimulus. In *c*, for stimulus interval 11 msec, the first relayed wave again diminishes, but when the interval shrinks in record *d*, the first relayed volley increases beyond its original value (in record *a*). It is to be noted that in all these records the conditioning stimulus is being successively shifted in towards the test stimulus. In record *e* the interval has decreased to 6–7 msec with the result that a new diminution of the amplitude of the first relayed volley occurs which in record *f* is succeeded by a third phase of increase in amplitude of this wave, all these effects being perfectly regular and reproducible. A third depression sets in at stimulus interval 3–4 msec in record *g*. The greatest facilitation of the first relayed wave is found in record *h* at interval 1–2 msec. In this record the second relayed wave again turns up so that the picture now looks like an amplified record *a*.

In record *i* the two stimuli are practically simultaneous. Both relayed waves are now depressed. The conditioning stimulus then passes the test stimulus (in *k*) and consequently the first relayed wave rises to its original amplitude. But the second relayed wave is so late that it still has a chance of being influenced by the conditioning stimulus and so reaches a final maximum in record *k* when the conditioning stimulus succeeds the test stimulus by 1–2 msec. The second relayed wave reaches a minimum in *l* and is indi-

s a particularly well synchronized relayed volley with latent period 3 msec. The first shock artifact comes from the popliteal conditioning stimulus that itself was without a visible effect. As this stimulus approaches the test stimulus in record *a* the large relayed wave diminishes and disappears com-

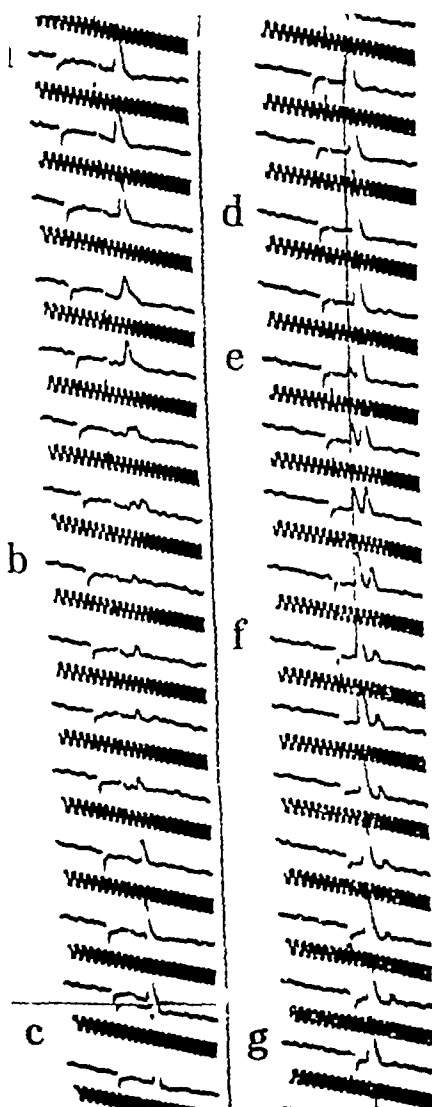


FIG 4 Records of relayed wave, elicited by test stimulus, influenced by conditioning stimulus at intervals shortening from *a* to *g* (see intervals between shock artifacts) See text

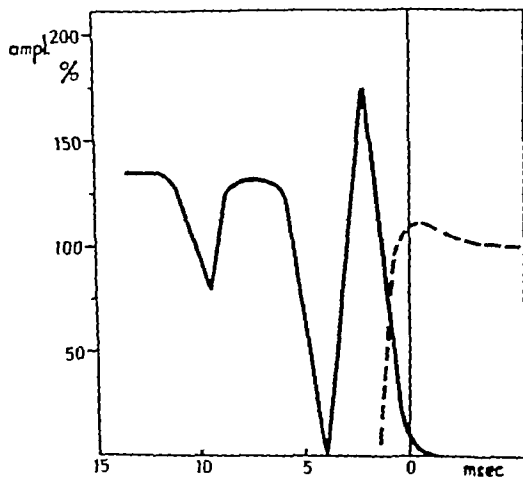


FIG 5 Diagram, plotted as in Fig 3, referring to 4 experiments of the type shown in Fig 4 Fully explained in text

pletely in record *b* at an interval of 4 msec. Further shortening of the interval is accompanied by a renewed increase of the relayed volley which in record *c*, at an interval of 2 msec, is greatly facilitated. From record *d* onwards follows a new depression of the relayed wave accompanied by a lengthening of its latent period. At the same time, from *e* onwards, there appears a new wave in front of it. The two waves

are about equal between *e* and *f*. From *f* to *g* the new wave has taken up the place of the original wave of record *a* whereas the second wave has diminished and finally disappeared in *g*.

Four similar experiments are collected in the diagram of Fig 5 set up as

Table 1

INHIBITION

FACILITATION

Expt No	I		II		III		IV		V		VI		VII	
	r	a	b	a	b	a	b	a	b	a	b	a	b	
1	2 3	1 5	3 8	4 8	7 3	8 3	10 6		3-4	5 3-6 3	5 5-7 5	7 8-9 8	10 -	12 3 -
2	3	1 9	4 9						2 5-4	5 5-7				
3	3	2 5	5 5			9 5	12 5	14	3-7	6 (-10)			9 5-12	12 5-15
4	3	2 5	5 5					16 19			5-8	8-11		
5	3	1	4	4	7	9 5	12 5	11 14	1 5-3	4 5-7	6-8 5	9-11 5		
6	3 2	1 5	4 7	5	8 2				2-4 5	5 2-7 7				
7	4										4-7	8-11		
8	4	0 5	4 5	3 8	7 8	7 8	11 8		1 3-2 5	5 3-6 5	4 5-7 2	8 5-11 2		
9	4 5	0	4 5	3 5	8			9 5 14	1 5-2 5	6-7	4-6	8 5-10 5		
10	4 5	-0 5	4	3 5	8	6.5	11	11 15 5	0 5-2 5	5-7	4 1-5 4	8 6-9 9	8-9 6	12 5-14 1
11	7 5	-3	4 5	0	7 5				-2--0 8	5 5-6 7	1-3	8 5-10 5		
Average			4 5		7 7		11 7	16 1		5 5-6 9		8 6-10 7		12 3-15 6

r, total latent period for the relayed waves obtained in different experiments

a, intervals in milliseconds between conditioning and test stimulus when the former caused maximal inhibition (I-IV) and maximal facilitation (V-VII) of the relayed waves elicited by the test stimulus

b = a + r in the different experiments

peroneal nerve. Inspection of the vertical columns *b* from the 11 experiments analyzed reveals the simple rule that the periods I-VII of facilitation and depression recur with remarkable constancy from experiment to experiment. The averages are found in the lowest horizontal column of the table. These show that inhibitory periods centre around latencies of 4.5, 7.7, 11.7, and 16.1, and periods of facilitation around 5.5-6.9, 8.6-10.7, and 12.3-15.6.

¹ All these periods have not been obtained in all experiments. Particularly complete is Expt. 10 in which

4 periods of inhibition and 3 periods of facilitation were seen, as illustrated by the table. It should further be noted that these periods occur in their fixed positions independently of whether the relayed wave studied in the experiment has had a long or short latent period *r*. The values for *r* range from 2.3-7.5 msec. Considering the variations that are likely to occur from experiment to experiment the constancy of the periods of facilitation and inhibition must be regarded as satisfactory.

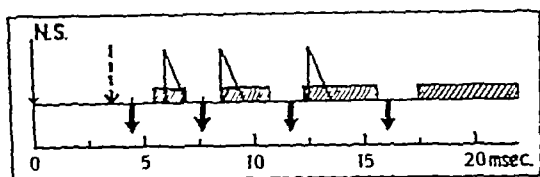


FIG. 6. Diagram illustrating the periodic variations of inhibition (arrows below horizontal) and facilitation (oblongs) following the conditioning stimulus (N.S.). The triangles show the temporal positions of waves elicited by the conditioning stimulus. Time from N.S. to first arrow = total peripheral condition time. See text.

The results of Table 1 are graphically summarized in the diagram of Fig. 6 which also supplements the analysis with a reduction to central reflex times. In this diagram the arrow at N.S. (zero abscissa) marks the moment of the conditioning popliteal stimulus. In the best experiments, as pointed out above, this stimulus elicits three waves, marked by triangles. The first of these waves is seen in most cases, the second and third less regularly. The second dotted arrow signals the moment of arrival of the conditioning volley into the spinal cord. The heavy arrows, pointed downwards, illustrate the lowest points of the troughs of inhibition, the oblongs the periods of facilitation of the relayed volleys, set up by the test stimulus. The diagram shows that these periods are placed in a definite temporal position relative to the moments at which the conditioning stimulus sets up a process in the centre. The first time this happens represents the moment of arrival of the first volley from the conditioning stimulus into the centre. This moment (the dotted arrow) can only be deduced by subtracting peripheral conduction times because no wave represents it at the recording electrodes on the efferent side. The following 3 waves (triangles) may or may not be recordable at the peroneal nerve. There is apparently a fourth relayed volley, elicited by the conditioning stimulus and noticeable in the diagram as a fourth oblong. The equivalent relayed volley is too small to be recordable. The central reflex times are represented by the distances from the dotted arrow to the various phenomena marked in the diagram.

Fig 3 The line drawn in full refers to the large relayed volley of record *a*, the broken line to the new wave turning up at brief intervals between conditioning and test stimulus The former curve has two inhibitions at 9–10 msec and 4 msec respectively The sharply delimited facilitation maximum at 2 msec is succeeded by the splitting up of the relayed response into two component waves and the final disappearance of the second one of them The first component (broken line) then gradually takes up the position of the original wave It is probable that the second component of the double wave at these time intervals actually is the facilitation of a phase of the conditioning response that at other intervals was subthreshold This facilitation must then have overlapped with the inhibition of the test response

4 *Analysis of time intervals of facilitation and depression* The typical experiments, described in the foregoing sections, have emphasized the extraordinary rapidity with which periods of facilitation and inhibition may succeed each other in the reflex activity of the spinal cord The precision necessary for an analysis of these changes has been attained with the aid of small shifts in the position of the photocell that controls the conditioning stimulus, towards and past the photocell activating the test stimulus to the spinal cord

A detailed analysis of the temporal properties of the periodic states of facilitation and depression reveals the existence of rules, masked in Fig 3 and 5, because in them the variations of excitability were plotted merely against intervals between conditioning and test stimulus In Table 1 these intervals are set down vertically in the columns *a* for the periods of facilitation or inhibition marked I, II VII The table summarizes 11 experiments (No 1–11) each of them presented horizontally with its own periods of facilitation and inhibition (I–VII) In each experiment the effects have been analyzed for one particular relayed wave The total latent period (*r*) of this relayed wave is to the left in the first horizontal column referring to the particular experiment in question Thus, for instance, there are five experiments (No 2–6) in which the latent period of the relayed wave is around 3.0 msec In the last experiment (No 11) a relayed wave following as late as after 7.5 msec has been analyzed The negative value (–3) in the first column *a* of this experiment signifies that the conditioning stimulus has succeeded the test stimulus by 3 msec, represented in Fig 3 by values to the right of zero abscissa In this case an effect of the conditioning stimulus upon the test stimulus is possible only in experiments where the relayed wave itself has had a relatively long latent period so that this stimulus, despite the negative value, actually has been effective *before* the relayed wave has occurred

From this follows that the theoretically significant figures in the table are not represented by the intervals *a* for the shock artifacts but by the sum of this interval *a* + the latent period of the relayed volley *r* This sum appears in the vertical columns *b* of Table 1 These figures represent the moments of appearance of facilitations or depressions at the recording electrodes on the

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DISCUSSION

The fact that the periods of facilitation and inhibition have such a strict temporal localization with respect to the central effects of the conditioning popliteal stimulus suggests that the region in which interference takes place has very small dimensions. The regularity could, for instance, be determined by a reverberation circuit (5, 12, 10, 11), a chain re-exciting itself and thus originating a volley at the same focal place after intervals differing relatively little from each other but increasing in length as activity in the chain gradually is damped out. Each wave of activity would be succeeded by a period of inhibition followed by a period of facilitation. In this manner Fig. 6 could be explained on the basis of the principle of relayed volleys. It is to be noted that the first inhibition of this figure precedes the first sign of excitation at the recording electrodes. A similar early inhibitory phase has been noted by Renshaw (14, see also 8). In Fig. 6 it can be explained, like the other inhibitory depressions, as a consequence of the arrival of the first volley of excitation to the critical region in the spinal cord.

But recent work by Granit and Skoglund (6) has suggested another possible line of thought. They have studied the properties of the "artificial synapse," formed by the cut end of a peripheral nerve (cats) in which impulses set up in the motor roots are relayed over into sensory fibres so that they can be picked up in the sensory roots. By testing with two successive stimuli ("conditioning volley" and "test volley") they found that this "synapse" sets up brief periodic states of facilitation and inhibition strongly reminiscent of those found above. This being so it is necessary to reckon with the possibility that secondary volleys also can be caused by similar periodic variations at a single synapse and thus need not be relayed in the manner hitherto assumed by all workers in this field including Bernhard and Granit (1).

SUMMARY

Local stimulation with needle electrodes placed laterally in the spinal cord elicits a number of oscillations of synchronized impulses which have been picked up in the peroneal nerve. The different synchronized waves of this complex response can be inhibited or facilitated by a conditioning stimulus to the ipsilateral popliteal nerve in a manner dependent upon the time interval between the conditioning stimulus and the test stimulus to the spinal cord.

It is shown that the conditioning stimulus causes a rapid periodic variation of inhibition and facilitation in which four inhibitory minima and four excitatory maxima can be noted. The number of such depressions and facilitations may vary from experiment to experiment but their temporal location with respect to conditioning stimulus is constant within relatively narrow limits.

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It is shown that the conditioning stimulus causes a rapid periodic variation of inhibition and facilitation in which four inhibitory minima and four excitatory maxima can be noted. The number of such depressions and facilitations may vary from experiment to experiment but their temporal location with respect to conditioning stimulus is constant within relatively narrow limits.

SUSTAINED FACILITATION AND POST-INHIBITORY REBOUND OF RELAYED VOLLEYS IN THE SPINAL CORD*†

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STIMULI applied locally with needle electrodes in the spinal cord, apart from exciting motor or sensory roots directly, may also elicit effects relayed over one or several synapses (2, 8). Direct and relayed volleys can be picked up, for instance, in the peroneal nerve and be conditioned by other stimuli in the usual manner. In a previous paper (1) the effect of a conditioning stimulus to the ipsilateral popliteal nerve was studied with the aid of test shocks applied to spinal cord electrodes and it was noted that the conditioning stimulus was succeeded by rapid periodic oscillations of excitation and inhibition, characterized by facilitation and depression of the relayed volleys picked up from the peroneal nerve. Generally four alternating periods of excitation and inhibition could be recorded. Their properties were described in the previous paper.

In this work will be shown in what manner the periodicity of the excitatory effect following a conditioning stimulus is related to the well-known phenomena of reflex rebound (11) and sustained facilitation (5). For details of technique and procedure, see the previous paper (1).

RESULTS

The periods of facilitation and inhibition. In the experiment, illustrated in Fig. 1-4, histological control showed that the electrodes had been placed at the level of 16 in the region marked in Fig. 1A. The test shock to these electrodes (insulated down to the tip) did not elicit any direct wave but only the relayed wave seen in Fig. 2a-b, Fig. 3b and Fig. 4a. The latent period of this wave was about 2.5 msec. The conditioning shock to the ipsilateral popliteal nerve elicited two successive relayed volleys with latencies 4.9 and 7.1 msec (triangles, Fig. 1B). At the amplification used in Fig. 3a only the first of these relayed waves is visible. The experiment was begun by localizing the time intervals between conditioning and test shock at which optima of facilitation and inhibition were present. The result of this preliminary experiment is found in diagram 1A in which the abscissa represents the shock intervals and the ordinates the height of the relayed volley, subjected to analysis, in relation to its normal amplitude 100 (conditioning stimulus absent). The diagram shows that the conditioning popliteal stimulus causes maximal facilita-

* Aided by a grant from the Rockefeller Foundation for neurophysiological research.

† Of necessity it has not been possible to have proof for this paper read by the author and many of the references have been supplied in the editorial office in order to make them complete.

tion, at a frequency of 9 per sec, the inhibitory interval I_1 of Fig 1B and 1A Figure 2 should then be consulted In 2a the effect of the test stimulus to the spinal cord with its first relayed volley is shown alone Repetition of this stimulus by itself leads to a gradual diminution of the effect which ultimately

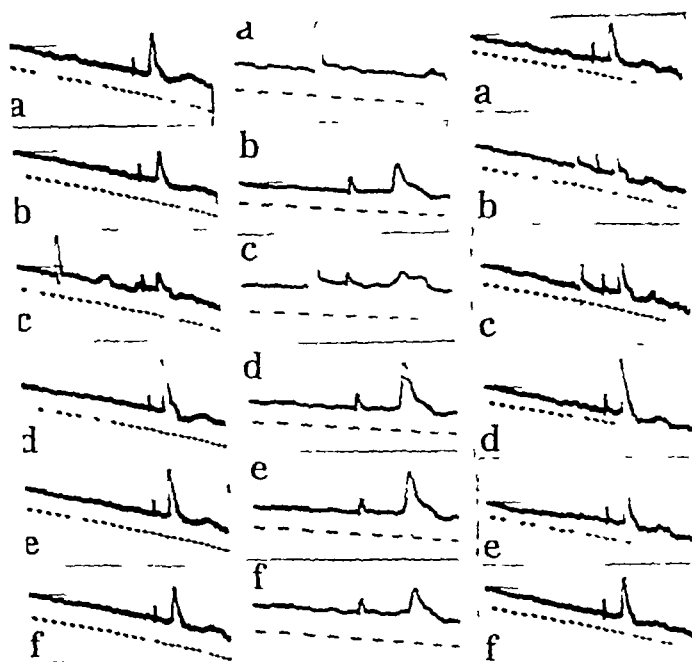


Fig 2

Fig 3

Fig 4

FIG 2-4 Responses from the recording electrodes on the peroneal nerve following stimulation of the homolateral popliteal nerve (conditioning stimulus) and the spinal cord (test stimulus) The pair of stimuli recur at a frequency of 9 per sec

FIG 2-3 Rebound (2 and 3d-f) after inhibition (2 and 3c) compared with effect of test stimulus (2a, b and 3b) before inhibition In Fig 2c interval between conditioning and test stimulus 11 msec (cf I_1 in Fig 1A), in Fig 3c, 1.5 msec (cf. I_1 in Fig 1A) 3a, effect of conditioning stimulus alone

FIG 4 Sustained facilitation (d-f) after facilitation (b-c) at interval 2.5 msec between conditioning stimulus and test stimulus (cf F_1 in Fig 1A) compared with effect of test stimulus alone (4a) See text

settles down to a constant value, shown in 2b When this constant amplitude has been reached the popliteal conditioning stimulus is introduced, as in 2c, and this combination is then repeated at a frequency of 9 per sec Figure 2c shows that the effect of the popliteal stimulus is inhibitory and this inhibition is noted during the whole time the experiment lasts (repeated combinations at 9 per sec) The popliteal conditioning stimulus is then discontinued

tion of the relayed volley from the spinal cord electrodes when it precedes it by 11.5, 7.5, 5.3 and 2.7 msec. The corresponding values for maximal inhibition are 9.0, 6.7, 4.0 and 1.0 msec. If to the latent period of the tested relayed volley are added the intervals at which facilitation or inhibition occur the total latent periods for the facilitatory and inhibitory periods are obtained in relation to the conditioning popliteal stimulus. They are then referred to the moment at which the effect is recorded in the peroneal nerve. A presenta-

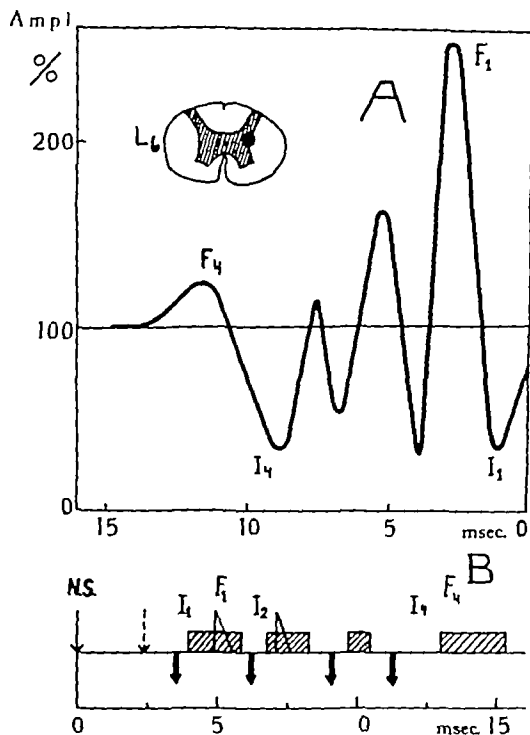


FIG 1 A Relative amplitude of a relayed wave (latency 2.5 msec), caused by test volley to spinal cord electrodes, is plotted against interval between conditioning stimulus (homolateral popliteal nerve) and test stimulus. See text. Inset: diagram of cross section of spinal cord at the level of L6 with localization of test stimulus electrodes marked as black spot.

B Diagram of values obtained from experiment, shown above in A, illustrating periodic variations of inhibition (arrows below horizontal) and facilitation (oblongs) consequent upon conditioning stimulus (N.S.), applied at zero time on the abscissa. The triangles show the temporal position of waves elicited by the conditioning stimulus alone. Time from NS to dotted arrow = total peripheral condition time. I_1-I_4 , periods of inhibition; F_1-F_4 , periods of facilitation.

tion on this basis of the same experiment is shown in Fig 1B (1). Four successive periods of facilitation and inhibition are seen to have been recorded in this particular experiment.

Inhibition and post-inhibitory rebound. Assuming now that in this particular experiment an interval between conditioning stimulus and test stimulus is chosen for which facilitation and inhibition is optimal. What, may we ask happens if such a pair of combined stimuli is repeated at the particular interval chosen? The experiment suggested by this question amounts to enquiring whether repetition of a given combination of time intervals between the two stimuli sets up any measurable after-effects and if these depend upon whether the interval chosen represents facilitation or inhibition.

In order to study facilitation there is a choice of four possible stimulus intervals and the same holds good for inhibition. Let us first select for repeti-

interval is 25 msec corresponding to the facilitation maximum F_1 in diagram 1a. The relayed volley is shown by itself in Fig 4a. In 4b and c the conditioning stimulus has been introduced and repetition of this combination again takes place at a frequency of 9 per sec. The relayed volley has been facilitated to nearly twice its original size. If then, after about 3 sec of repetition of this combination the conditioning stimulus is discontinued, its effect still lingers on, as shown by 4c and d, to disappear after about 5 sec at which moment 4f is recorded.

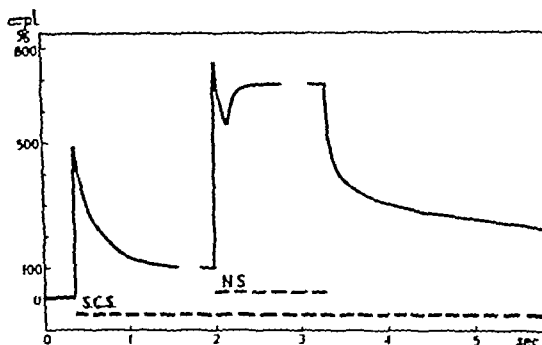


FIG 6 As Fig 5, but in this case the test stimulus (NS) causes facilitation, gradually disappearing after withdrawal of the test stimulus (sustained facilitation). See text.

A similar experiment is presented graphically in Fig 6, set up as Fig 5. In this case the relayed volley had a total latent period of 35 msec, the interval between conditioning and test shock being 10 msec. This means that the period of facilitation analyzed was the last one, F_4 of diagram 1B, i.e., $35 + 100 = 135$. The popliteal stimulus (NS) augmented the relayed volley to just above 7 times its

original stabilized value. The sustained facilitation lasted about 5 sec after cessation of the conditioning stimulus (NS). If the conditioning stimulus (SCS) alone had been repeated at the same frequency of 9 per sec the relayed volley would have disappeared the moment stimulation was stopped.

In the two experiments described the facilitating effect has been studied for the first and fourth facilitation period (F_1 and F_4 of Fig 1B). But sustained facilitation has also been found for the second and third period and with relayed waves of different latent periods. Similarly the third inhibitory period has had the properties described above for the inhibitory periods I_1 , I_2 and I_3 . In all experiments the stimulus frequency has been low, below 20 per sec. The effect of frequency will be subjected to a special analysis.

In order to avoid complications from direct waves the stimulus to the spinal cord has generally been applied in such a manner that direct volleys were avoided. The location illustrated in Fig 1A seems to be particularly favorable from this point of view. The histological control has shown that relayed waves of different latent period are most easily obtained when the electrodes are placed in the grey matter. By avoiding the anterior and posterior horns direct waves are easily avoided.

DISCUSSION

In earlier experiments on reflex muscular contractions rebound contractions have been noted after both ipsilateral and contralateral flexor reflexes (10) and possible explanations have been suggested, in particular by Sher-

but the test stimulus to the spinal cord is still repeated at the same frequency. Figure 2d shows that the immediate effect of removal of the inhibitory conditioning shock is an increased amplitude of the volley which now exceeds its original value in 2a. Gradually this post-inhibitory facilitation vanishes (2e) and in 2f the amplitude of the relayed volley has returned to its original level as given by 2a.

The experiment has thus shown inhibition and post-inhibitory rebound (for the relayed volley) by repetition of the combination representing the last inhibitory period I_4 of diagrams 1A and B. In Fig. 3a similar effect is shown on the same relayed volley (latency 2.5 msec) but in this case the combination used corresponds to the *first* inhibitory period I_1 of Fig. 1B. In

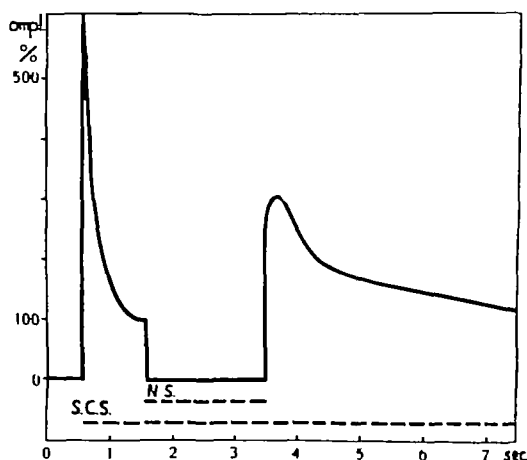


FIG 5 Relative amplitude of a relayed wave, caused by repetitive test volleys to spinal cord (S.C.S.) As shown by broken lines above abscissa, conditioning stimulus (N.S.) appears for a brief duration in the middle of the record. The test stimulus is repeated the whole time, from S.C.S. onwards. During stimulation with the conditioning stimulus (N.S.) there is inhibition, succeeded by rebound afterwards. See text.

3a is shown the effect of the conditioning popliteal stimulus alone, in 3b the relayed test volley alone, in 3c the inhibition caused by combining conditioning the test shock, in 3d and e the rebound after removal of the conditioning stimulus, and in f, finally, the return to the original value after disappearance of the rebound facilitation.

A graphical presentation of the outcome of a similar experiment is found in Fig. 5 referring to a relayed volley with the latent period 3.5 msec and an interval for inhibition corresponding to I_2 of Fig. 1B. The ordinate is the relative size of this volley and 100 corresponds to the semi-stationary state reached after some time of repetition of this stimulus (S.C.S.) alone. The abscissa illustrates duration of stimulation in sec. N.S. represents the duration of the conditioning stimulus to the nerve. In this particular experiment there was complete inhibition of the relayed volley and the rebound augmentation of this volley rose to three times the original stabilized value of 100. After cessation of the N.S. stimulus it lasted 7–8 sec. before this value was re-established.

Sustained facilitation. An interval between conditioning and test shock at which facilitation occurs is studied in a similar manner in Fig. 4. This

MIDBRAIN AUDITORY MECHANISMS IN CATS*

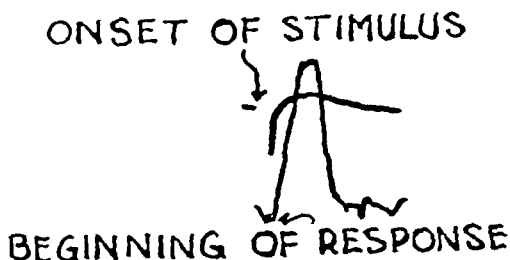
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THERE ARE available several descriptions of the nuclei and tracts which deal or are presumed to deal with impulses reaching the brain via the cochlear nerve. With few exceptions, these are concerned entirely with anatomical connections and are based on the study of normal material variously stained by Nissl, myelin sheath or silver impregnation methods. Only recently (1) there appeared a study of ascending auditory tracts from Marchi degeneration after brain stem lesions. Aside from this, the usefulness of the extant in-

FIG 1 Photograph of cathode ray trace showing the split beam with onset of stimulus indicated by break in upper trace, lower trace shows response to single click stimulus



formation has been most meagre in the interpretation of auditory function; this impressed us particularly in a recent attempt to account anatomically for the retention, alteration or loss of learned auditory responses after systematic surgical invasion of the auditory pathway (3). The difficulty is that there is simultaneously too much and too little information. For example, minute descriptions of the configuration of the cochlear nuclei are to be found, but none gives any hint of functional differentiation even between dorsal and ventral cochlear nuclei, much less between the numerous areas of specific cytological structure described by Lorente de Nó †. On the other hand, data on the connections of brain stem nuclei seem inadequate to permit one accurately to account for retention of learned auditory responses at mid-brain or medullary levels.

It is the purpose of this paper to re-examine certain data already published, present some new data, and appraise the status of these aspects of auditory function.

METHOD

Responses to audible, thyratron-generated clicks delivered one foot from the ear of the experimental animal (cat) were recorded from various points in the midbrain by means of a single-phase, capacity-coupled amplifier and photographed from the face of a cathode-ray oscillograph. The beam was split by an electronic-switch arrangement, response appearing on one of the resultant traces, stimulus on the other (Fig. 1).

* Contribution number 59, Laboratory of Psychology, University of Rochester. This investigation was aided by grants from the Research Council of the American Otological Society and the Research Committee, University Center in Georgia.

† The only difference in the connections of the dorsal and ventral cochlear nuclei according to Barnes, Magoun and Ranson (1) would seem to be the precise route of crossing of the secondary fibers.

rington (12, see also 4, 7, 10) The experiments described in this paper add a new factor by pointing to the significance of proper timing of the interacting stimuli and correlating the phenomenon with the inhibitory phase of the periodic variations following a stimulus to the center At the same time they confirm Sherrington's original view that rebound is a consequence of preceding inhibition

In the earlier reflex work rebound was sometimes noted without preceding inhibitory relaxation (4, 11) and assumed by Graham Brown to be "concealed" Similarly, in this work, it has been noted that rebound occurs after stimulation with a combination *representing an inhibitory interval*, even when the actual inhibition has been slight or absent It is clear that an average reaction such as a reflex muscle contraction must then be able to exhibit rebound without evident signs of a preceding inhibition Nevertheless the necessity of using an inhibitory interval between conditioning and test stimulus in order to elicit this phenomenon demonstrates the essential place of pre-excitatory inhibition in the rebound reaction

It may be of interest to point out that the retinal off-effect in a like manner presupposes pre-excitatory inhibition (8) and that recently Bremer (3) has shown that there is a similar off-effect or rebound in the acoustic area of the cat

The sustained facilitation, studied by Eccles and Granit (5) in both contralateral and ipsilateral reflexes also is a quite regular phenomenon, referable to the stimulation intervals at which periodic facilitation occurs and can be analyzed in the manner described

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reduced. Severance of the commissure now eliminated the remaining potentials except for occasional equivocal deflections as in Fig 2, 12, histological examination leaves a slight doubt as to completeness of the section of the left lemniscus, and it is possible a few fibers may still have been discharging into the colliculus

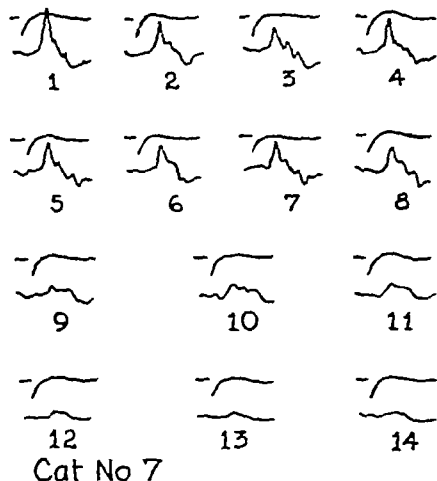


FIG 2 1-4, response of left inferior colliculus to click stimulation, 5-8, response from same electrode placement after destruction of left cochlea, 9-11, response from same placement after severance of left lateral lemniscus, 12-14, response from same placement after severing commissure of inferior colliculus

Cat 9 represents a repetition of the previous experiment, except that in this case the contralateral (right) cochlea was destroyed as the first operation. This resulted in a marked consistent reduction in amplitude of response (Fig 3, 5-8). In this instance the diminution may be more profound than in

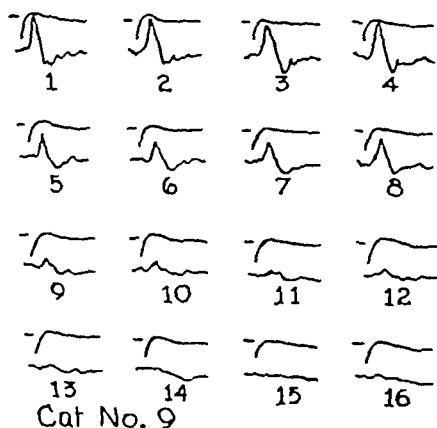


FIG 3 1-4, response of left inferior colliculus to click stimulation, 5-8, same after destruction of right cochlea, 9-12, same after severance of left lateral lemniscus, 13-16, same after severance of commissure of inferior colliculus

cat 7, though comparison of the two figures leaves some doubt, however, it is probably safe to say there is evidence of greater potency of the contralateral ear over the homolateral in production of the collicular response, although this difference is not sufficient to encourage one to believe it might be expressed in functional determination of an audiogram for example. Section

In most experiments, the silver wire, unipolar electrode was left in place throughout, recording potentials first in the intact animal, and, after appropriate surgical elimination of one or more auditory tracts (as detailed in the individual protocols), recording potentials again from the same electrode placement to determine resulting changes. In a few cases, the electrode was moved from placement to placement as occasion demanded.

Brains of all cats were removed at the conclusion of the experiments, fixed in formalin, and sectioned serially by the paraffin method to permit determination of the location and extent of lesions.

RESULTS AND DISCUSSION

Secondary auditory tracts Barnes, Magoun and Ranson (1) by tracing degeneration paths from lesions in dorsal or ventral cochlear nuclei, or both, found that all the fibers leaving these nuclei cross the raphe to ascend in the contralateral lateral lemniscus or terminate in the superior olivary complex, fibers from the dorsal nucleus cross in and compose the dorsal and intermediate striae medullares of Monakow and Held, while the ventral nucleus sends some fibers through the stria of Held, but most of them through the trapezoid body. In either case the fibers turn sharply rostrad to ascend as the principal components of the lateral lemniscus. Many fibers could be traced rostrally to terminations in the inferior colliculus, none ascending higher, but considerable numbers also terminate in superior olivary complex or nucleus of the lateral lemniscus. Barnes, Magoun and Ranson indicate no difference between secondary paths from dorsal and ventral nuclei except the choice of the bundle in which crossing takes place.

From their experiments it is apparently uncertain whether or not any fibers may terminate in the homolateral superior olivary complex, although the authors regarded it as probable. This question will be discussed relative to that of the bilateral projection of auditory impulses.

Commissure of the inferior colliculus Cats 6, 7 and 9 illustrate three experiments carried out to determine the approximate proportion of (inferior) collicular response attributable to conduction over the inferior collicular commissure, and, incidentally, to provide some index of the relative contribution of homolateral and contralateral ears to collicular response.

In the case of cat 6, responses were first recorded from the left inferior colliculus. The commissure was then severed with the result that the collicular response was just perceptibly reduced in amplitude. The left acoustic nerve was then sectioned. This operation had the effect of still further reducing amplitude of response, but this time by approximately fifty per cent. Finally, as a check, the left lateral lemniscus was severed, upon which collicular response was entirely abolished.

In cat 7, after recording the normal response from the left inferior colliculus, the homolateral cochlea was destroyed, after this procedure the colliculus received impulses through the left lemniscus only from the contralateral ear, and, also, via the right lemniscus and commissure from the same source. The response was reduced in amplitude on the average, but the attenuation was by no means severe (see Fig 2, 5-8). When the left lateral lemniscus was severed, leaving the commissure as the only route by which impulses could reach the left inferior colliculus, the response was greatly

Table 1 * Changes in absolute intensity limens following bilateral removal of the inferior colliculus

Cat	125	Frequencies 1000	8000 c.p.s
19	-15 7	-18 7	+ 1 3 decibels
20	-14 1	-14 0	- 6 4 decibels
22	-21 6	-17 0	-23 0 decibels
27	-10 0	-12 5	-10 0 decibels
28	-16 3	-11 2	-33 3 decibels

In all of the animals listed in Table 1, with the exception of cat 20, destruction or disconnection of the inferior colliculi was complete bilaterally, and in all of them it was considered that some bypassing fibers of the lateral lemniscus probably escaped being severed, on one or both sides. Cat 20 sustained complete destruction of the left colliculus and its brachium, but only minor damage was found in the right colliculus, namely a relatively small lesion situated medially.

* Kryter and Ades, 3, p 522

that even the few direct olivo-geniculate fibers described are minimized by the authors. In the experiments of Kryter and Ades (3), several of the animals which had been subjected to bilateral collicular destruction showed definite signs of retention of at least some functioning connections above mid-brain level. Table 1 is reproduced directly (3, p 522, Table II). These results may be compared with those set forth in Table 2 which represents functional hearing changes in animals with auditory pathways sectioned below the collicular level.

Table 2 Changes in absolute intensity limens following bilateral elimination of midbrain auditory connections

Cat	125	Frequencies 1000	8000 c.p.s
1	-33 1	-47 4	-51 0
4	-37 7	-46 5	-51 7
16	-45 3	-35 0	-25 2
20	-23 3	-47 2	-61 9
26	-25 8	-26 1	-47 4

Histological inspection of the brains of the cats listed in Table 2 showed that as a result of one or a combination of several operations the midbrain of each cat was ultimately deprived of all or nearly all connections through which it might mediate auditory reflex. For further details on these animals, please see pp 523-527, Kryter and Ades (3).

It is apparent, when the two groups represented in Tables 1 and 2 are compared, that the functional changes are of different order. We attempted to explain this on the following basis. The first group, while losing midbrain auditory function through destruction of the inferior colliculi, retained residual connections to the medial geniculate (and, therefore, to the auditory cortex) on one or both sides, the cats of the second group, after final operations, had no remaining functional connections in the midbrain or above that level. If these observations are correct, the only explanation of the

of the left lemniscus again had a profound effect on the collicular response, reducing it by much more than half, while subsequent section of the commissure eliminated this small residual response

It is dangerous too readily to apply data of this sort in the interpretation of normal auditory function. Nevertheless, it is certainly fair to assume some correlation between electrical activity of neural tissue and its functional potency, particularly where experimental differences are as large and obvious as they are in these experiments. Consequently, the conclusion seems justified that the commissure of the inferior colliculus plays but little part in accounting for functional bilateral equality of auditory projection. It is difficult to believe the slight potentials conducted by the commissure could represent a significant degree of activity in normal auditory function. Similarly, the slight differences in homolateral and contralateral contribution to collicular response in cats 7 and 9 may be taken to indicate a virtual bilateral equality.

Homolateral auditory projection. Barnes, Magoun and Ranson (1) deny the possibility of homolateral ascending fibers from the cochlear nuclei. This immediately calls into question the results of several experiments performed in our laboratory over the last ten years, including those of Mettler *et al* (4), Brogden (2) and those of Kryter and Ades (3) more recently. Both series of experiments indicate at least functional bilateral equality of representation of both cochleae in the cerebral cortex. Barnes *et al* offer as a possible explanation the crossing of auditory impulses in the commissure of the inferior colliculus and at that of the nucleus of the lateral lemniscus (Probst's commissure). The collicular commissure has been explored electrically in the present study. The protocols and figures of cats Nos. 6, 7 and 9 indicate that the contribution of commissural discharge to the collicular response is of unimpressive proportions. Probst's commissure is anatomically a far less substantial bundle of fibers than the commissure of the inferior colliculus. If these small tracts are sufficient to account for the functional bilateral equivalence in the experiments of Mettler *et al* and those of Kryter and Ades, the "safety factor" thus expressed, and mentioned by Mettler, must be great indeed, if all auditory tracts were of proportionate potency, it would seem the system must be functionally nearly indestructible.

A more likely explanation is possible. Although there are no data which definitely indicate whether or not fibers from each cochlear nucleus terminate in both superior olivary nuclei, Barnes, Magoun and Ranson thought it probable that such bilateral terminations are present. If we assume that this is the case, all of the functional evidence for bilateral representation is immediately explained since a large proportion of the lateral lemniscus consists of ascending fibers from the homolateral superior olive.

Direct olivo-geniculate fibers. Barnes, Magoun and Ranson (1) reported that only a few of the fibers ascending from the superior olivary nucleus, and none from the cochlear nuclei, pass directly to the medial geniculate body without synapse in the inferior colliculus. One receives the impression

the left superior colliculus and from the brachium of the inferior colliculus, the inferior colliculus was undercut and partially removed, an effort being made to leave the laterally-lying fiber lamina of lemniscus and brachium intact while eliminating the collicular gray matter. Following this procedure, potentials from the superior colliculus were severely diminished, but not abolished. It was also noted that the brachium response remained about as strong as before. A second incision similar to that in the previous experiment

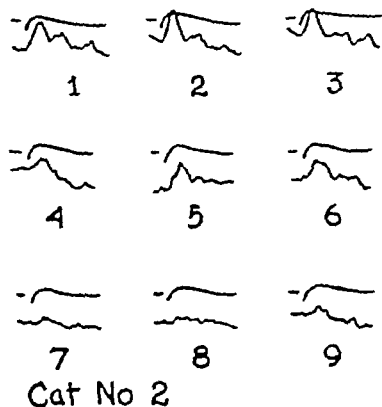


FIG 4 1-3, response of left superior colliculus to click stimulation, 4-6, same after vertical knife cut between left inferior and superior colliculi, 7-9, same after cutting between superior colliculus and inferior quadrigeminal brachium

was again required completely to eliminate the residual response from the superior colliculus. Other animals yielded substantially similar results under similar conditions.

Barnes, Magoun and Ranson describe three destinations for fibers leaving the inferior colliculus: (i) medial geniculate body of either side, (ii) the opposite inferior colliculus, and (iii) the pontine gray. In view of the absence of other connections, they take the view that the inferior colliculus, unlike the superior, does not function as a reflex center.

In all cats examined it was possible to record strong potentials from the surface or depth of the superior colliculus in response to audible clicks. These are not weak or equivocal responses, but are regular and of magnitude second only to auditory responses from the inferior colliculus and the auditory cortex. It may be argued that they are the result of direct spread from the closely adjacent inferior colliculus, an argument which may be met by the consideration that the auditory response of the superior colliculus is greatly diminished by cutting between the inferior and superior colliculus. This implies strong connections between the two nuclei. The literature contains occasional mention of fibers leaving the lateral lemniscus to terminate in the superior colliculus. Our evidence indicates that such fibers probably do exist, however, as may be seen from the figures, their functional significance is minimal and they are obviously only in small part responsible for the generous potentials recorded from the intact superior colliculus.

In the experiments of Kryter and Ades it was shown in several cats that auditory lesions above and below (or including) the inferior colliculus have

difference in degree of auditory loss is that either midbrain or geniculocortical mechanisms may suffice to maintain auditory threshold at a near-normal level in the absence of the other, but when both are eliminated, the threshold rises sharply. This, in turn, implies that a functionally significant residue of supratectal (*i.e.*, to medial geniculate) conduction is possible even after removal of the collicular synapses. This argues for a substantial number of lemniscal fibers which bypass the inferior colliculus. Admittedly, this is a difficult histological determination to make, and it would be most unsafe to say that collicular synapses did or did not remain functional in the brain of a given animal. Nevertheless, the animals designated in Table 1 had in common much less severe hearing losses than did animals with only medullary auditory circuits intact. They also shared the common histological finding of bilateral collicular removal with more or less undamaged tissue remaining on the lateral surface where the lemniscus and the brachium form a fiber lamina. There is some corroboration in animal 3 of the present series, which showed retention of auditory response in the inferior quadrigeminal brachium after destruction of the colliculus, this was also true of other animals in which the effort was made to remove collicular gray while sparing the lateral fiber lamina.

The weight of evidence, then, despite one's hesitancy to insist upon the histological observation, favors the conclusion that the direct olivo-geniculate component of the lemniscus alone is of sufficient proportions to account for functionally significant conduction above the midbrain even though most of the lemniscal fibers synapse in the colliculus. One may point out the additional finding that in the group of animals of Table 1, there are frequency-differential losses, which is what would be expected if varying proportions of bypassing fibers remained intact and if these differ among themselves with respect to frequency.

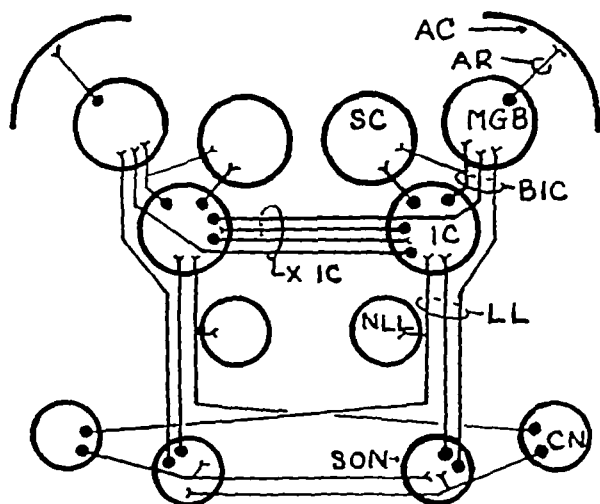
Reflex function of inferior colliculus and intercollicular conduction. Cat No 2. Preoperative potentials were recorded from the surface of the left superior colliculus (Fig. 4). A vertical knife-cut to the depth of the tectum was then made at the junction of superior and inferior colliculi. The amplitude of potentials from the superior promptly dropped to an extent illustrated in Nos 4, 5 and 6, Fig. 4. It was necessary to extend the cut anterolaterally in the lateral edge of the superior colliculus before the potentials from it could be completely eliminated. It is interesting to note that throughout the experiment, the level of spontaneous discharge from the superior colliculus was maintained without much change, despite the partial circumcision of the nucleus and disappearance of evoked auditory potentials. This was not true of the experiments in which, potentials being recorded from the inferior colliculus, that nucleus was gradually severed from its sources of afferent impulses, as the evoked potentials diminished, the spontaneous discharge also decreased, until, in the end, the base-line was smooth and flat (see Fig. 2 and 3).

Cat No 3. In this experiment, after recording normal potentials from

response to pure tone, functions as effectively as the cerebral cortex. For this function, it depends upon a pathway for discharge through the superior colliculus. When the inferior colliculus is destroyed or its discharge pathway is interrupted in the absence of the auditory cortex, the remaining "auditory" integration becomes most meagre and primitive.

Summary of connections of the central auditory pathway Figure 5 is a diagram of the connections of the auditory pathway so far as they can be deduced from the foregoing evidence. Neuron I, of course, is situated in the spiral ganglion of Corti and sends its axon via the cochlear nerve to termi-

FIG 5 Diagram of central auditory pathway. AC—acoustic cortex, AR—auditory radiation, BIC—brachium of inferior colliculus, CN—cochlear nucleus, IC—inferior colliculus, LL—lateral lemniscus, NLL—nucleus of lateral lemniscus, SC—superior colliculus, SON—superior olivary nucleus, XIC—commissure of inferior colliculus.



nate in the cochlear nuclear complex. Secondary neurons may cross and ascend as components of the contralateral lateral lemniscus to terminations in the inferior colliculus. Secondary fibers may also leave the cochlear nuclei to terminate in the superior olivary nucleus, both homo- and contralaterally.

Tertiary fibers leave the superior olivary nuclei, ascend in the homolateral lateral lemniscus and terminate either in the inferior colliculus or the medial geniculate body. Other third-order axons leave the colliculi via the inferior quadrigeminal brachium (where they are joined by those from the superior olives) to terminate in the homolateral medial geniculate, still others leave the colliculus via the inferior quadrigeminal commissure to terminate in the opposite colliculus or pass through to join the brachium. Finally, third order connections via histologically indistinct pathways make connections with the superior colliculus, at which place auditory impulses have reached a point at which they may be translated into motor impulses through the mediation of descending tracts from the superior part of the tectum.

Summarizing the cortical projection pathway thus far, third-order neurons from homolateral and contralateral inferior colliculi and from homo-

profoundly different effects on both qualitative retention of learned auditory responses and absolute intensive thresholds. In addition, it was found in the same series of experiments that removal of the superior colliculus and removal of the inferior colliculus have substantially the same effect on retention of such responses, which we take to be further evidence that the inferior colliculus discharges through the superior

It is customary to assign strictly visual function to the superior colliculus, and strictly auditory to the inferior colliculus, although it has long been taught that the descending pathway from the latter leads in some fashion through the former to discharge through tectospinal and tectobulbar tracts. It is hardly surprising, then, to find the superior colliculus responsive to auditory stimulation, and the conclusion seems inescapable that auditory integration at midbrain level is participated in by the entire tectum rather than by the inferior colliculus alone. Moreover, the functional evidence suggests integration of a fairly high order.

Without invoking the trite, but time-honored plea of the capriciousness of the *Marchi method*, it may be possible to account for the disparity in viewpoint on the functional significance of the inferior colliculus. Assuming the adequacy of the method, Barnes, Magoun and Ranson have shown only that this nucleus has no myelinated efferent fibers sufficient to account for reflex function. Our evidence as clearly indicates (1) that significant communication exists between it and the superior colliculus and (2) that the presence of the inferior (and, indeed, the superior) is essential to the maintenance of certain response-patterns to auditory stimulation. No great stretch of imagination is required to picture unmyelinated fibers passing between these two contiguous nuclei in considerable numbers, either organized into discrete bundles or scattered. We are unable to discover convincing histological evidence for discrete bundles, however, in the tectum, as elsewhere in the central nervous system, silver-impregnation reveals an abundant neurophil composed largely of unmyelinated fibers, which might well account for intercollicular conduction. The time is past when it was safe to evaluate the function of a mass of gray matter by determination of its myelinated connections alone. We have been too long bound to the tacit assumption that the large myelinated fiber bundles are all-important in the interpretation of neural function.

Again, support may be found in our previous experiments for the reflex significance of the inferior colliculus. It was found that as long as either the inferior colliculus or the auditory cortex of one side remained functional, the absolute intensity threshold dropped no more than 15 decibels on the average and the conditioned response was retained with no perceptible qualitative change. But immediately upon bilateral severance of the auditory pathway below the inferior colliculi, the threshold rose by an average of 40 decibels.

Thus, we arrive at a concept of the colliculus as a reflex mechanism which, at least for the relatively simple auditory integration of conditioned

PARALYSIS WITH HYPOTONICITY AND HYPER-REFLEXIA SUBSEQUENT TO SECTION OF BASIS PEDUNCULI IN MONKEYS

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THAT SPASTICITY is a characteristic feature of the syndrome of paralysis developing subsequent to lesions interrupting the upper motor neuron or cortico-spinal system is a long-standing concept of clinical neurology (1, 14). However, results of selective destruction of the cortico-spinal system in the monkey are beginning to modify this view (9). In a recent discussion of this subject Walshe (15) considers the possibility that the path, interruption of which adds the component of spasticity to hemiplegia, although cortical in origin, is a short one going to subcortical centres and not to the spinal cord.

In monkeys Hines (6, 7) localized a region in the precentral cortex between areas 4 and 6, the destruction of which leads to transient spasticity. Histologically this area is transitional between areas 4 and 6 and presents no delineating features. The identification of this "strip area" thus hinges on its inhibitory influence upon stimulation (3) and on the characteristic neurological signs of spasticity evolving from its ablation.

Evidently the projection from this cortical inhibitory area courses outside of the cortico-spinal tract, at least from the pyramid caudally, for Tower (13) sectioned the bulbar pyramid in the monkey and obtained essentially a flaccid paralysis. Furthermore, flaccid paralysis has been shown to follow interruption of the cortico-spinal tract within the spinal cord of the monkey (2).

Recent studies involving excitation of this cortical suppressor area indicate that its effects are mediated through the caudate nucleus (3, 10). Further information concerning the course of its projection might be gained by observing the effect of destruction of efferent cortical paths at levels between the cortex and the bulbar pyramid. One possible site is the basis pedunculi. The effect of section of this in the monkey forms the subject of the present report.

MATERIALS AND METHODS

In each of six monkeys the cortical projection fibers were interrupted within the right or left basis pedunculi. The operative procedure consisted of exposing the cerebral hemisphere sufficiently to retract the occipital lobe. The approach to the basis pedunculi was made along the superior surface of the tentorium cerebelli and the oculomotor nerve was located as it coursed forward from the ventral surface of the midbrain. A cut was inflicted lateralward across the basis pedunculi at the level of the oculomotor root.*

One of the monkeys (C-33) was sacrificed two and one-half months postoperatively, another (C-88) died of round worm infestation one month after operation and a third ani-

* This approach is similar to that used by Economo and Karplus in 1909 (4).

lateral superior olive project upon the medial geniculate body, from which, in turn, fourth-order neurons project to the auditory cortex

No attempt has been made in the diagram to indicate relative numbers of the various components. It will also be noted that the nucleus of the lateral lemniscus has been ignored, along with its commissure, except to indicate a somewhat problematical collateral connection from the lateral lemniscus. No indication is made of reflex connections below midbrain level, although one must strongly suspect that some such fibers exist in view of the functional indications and despite lack of anatomical evidence. Otherwise, the diagrammed connections are sufficient to account for the functional phenomena discussed.

SUMMARY

1 The contribution of discharge through the inferior collicular commissure is negligible in the total activity of the inferior colliculus

2 As measured electrically, the contribution of the contralateral ear to collicular response is slightly greater than that of the homolateral ear

3 The explanation of functional bilateral equality in auditory conduction from either ear probably lies in bilateral terminations of secondary auditory fibers in the superior olivary nucleus which contributes substantially to the lateral lemniscus

4 Functionally significant numbers of fibers of lateral lemniscus bypass without synapsing in the inferior colliculus

5 The inferior colliculus discharges through the superior colliculus

6 The inferior colliculus is an important reflex center capable of auditory integration

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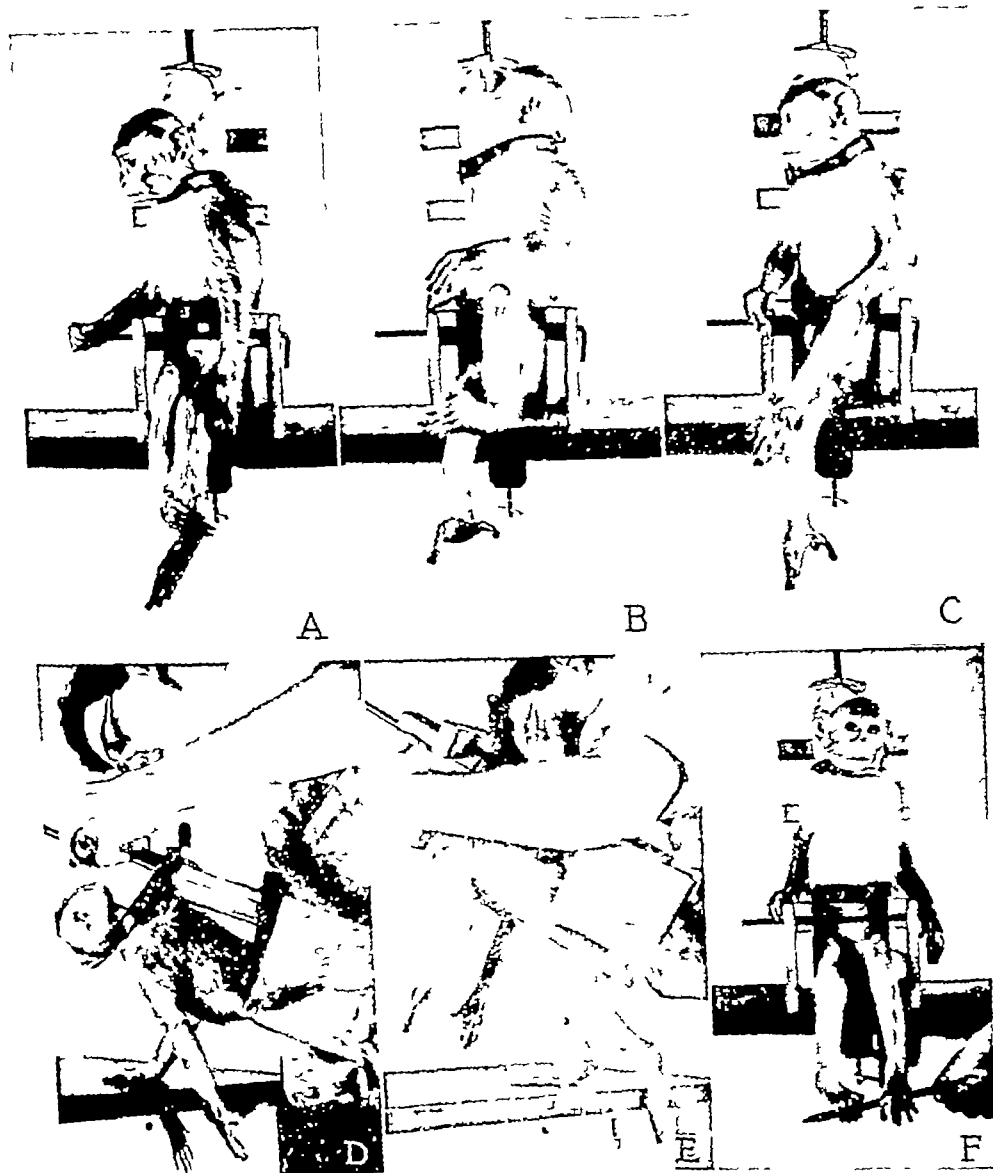


FIG 1 A-F Photographs illustrating various aspects of the symptomatology of Monkey C-73 after section of the right basis pedunculi, shown in Fig 2

Posture As seen in Fig 1A there was sagging of the left shoulder. The arm hung down and the left hand was apparently useless. The left leg and foot hung flaccidly. The right foot frequently grasped the affected leg. The digits of the left foot appeared extended. The monkey showed also a loss of the positive and negative supporting reactions of the lower left extremity. Tests for postural rigidity gave negative results.

mal (C-1) died four months after operation as a result of a second operation performed on the remaining, intact basis pedunculi. The other three animals (C-73, C-100 and C-90) were under observation from six to twelve months before being sacrificed.

Postmortem studies revealed severance of all but the most medial fibers of the basis pedunculi in five monkeys. The proportion left intact varied from the fibers intersected by the roots of the oculomotor nerve to as much as the medial one-fifth of the basis pedunculi. In the sixth monkey (C-1) a substantial lemniscus of fibers adjacent to the substantia nigra was also uninjured.

In one monkey (C-90) the cortical "strip area," 4s, was removed on the same side as the basis pedunculi operation six weeks following the latter. In another monkey (C-55) the peduncle was not sectioned but extirpation of area 4s was carried out to provide an animal with the syndrome of spasticity. The extirpation of cortical tissue was accomplished by suction.

Neurological examination of all monkeys were recorded daily during the early post-operative period and less frequently later on. Examinations were made from time to time by clinical neurologists who were invited to observe the animals without knowledge of the nature of the operation performed.† At each examination the monkeys were placed in a chair constructed to eliminate difficulties in handling.

RESULTS

The results of the neurological examinations were similar in five of the six monkeys with unilateral section of the basis pedunculi. The responses to the tests are presented in Table 1 and fall into two categories, those representing the signs of paralysis and those representing the signs of spasticity.

It was noted that the affected upper and lower extremities hung flaccid and that there was a palpable flabbiness of the muscle groups. Atrophy, evident about two weeks following operation, gradually became more noticeable. On passive manipulation, diminished resistance to movement in all directions was apparent. Grasping, placing and hopping were conspicuously absent in the affected extremities. Plantar and palmar stimulation yielded diminished responses or none at all, the plantar response being the more consistently absent. These phenomena along with the loss of voluntary motor power depicted a characteristic hemiplegia, the paralysis residing in the side opposite the lesion.

The quality of this paralysis resembled neither a typical spastic hemiplegia nor a purely flaccid paralysis. Some characteristics of release phenomena, notably the hyperactivity of tendon reflexes present in the paralyzed upper and especially the lower extremity, were observed. A rather marked extension of the digits was also a constant finding and suggested the presence of hypertonicity in the most distal muscle groups. The conclusive hypotonicity noted on manipulation of all other parts of the affected extremities and the absence of clonus, however, were characteristic of the flaccid type of paralysis.

A detailed description of one monkey (C-73) will serve to present the typical effects of a unilateral lesion. The right peduncle of this animal was transected. Observations and examinations were carried out daily for two weeks, weekly for the next twenty weeks and monthly thereafter until the animal was sacrificed one year postoperatively.

† To Dr Lewis J. Pollock and to Dr Erich Liebert, of the Department of Nervous and Mental Diseases, the authors extend thanks.

adductor muscle group of the right thigh. Frequently this crossed adductor response was obtained by striking the tibia of the left leg well below the knee, also the active suprapatellar reflex, not usually obtainable in the normal monkey, was an accompanying feature of the hyperactive patellar reflex. Less obvious was the exaggerated ankle jerk, which was not as constant and could only be noted by careful examination.

In the left upper extremity the tendon reflexes were more difficult to test. This might be explained by the failure to obtain as complete relaxation as was desirable. However, the deep reflexes—biceps, triceps and radio-periosteal—gave active responses more readily than in the corresponding contralateral extremity. In another animal, monkey C-33, when sedation was induced by intraperitoneal injection of nembutal, these deep reflexes of the upper extremity characteristically were found to display hyperactivity.

The phenomenon of clonus was absent in the left extremities and was never noted in those of the right.

In eliciting an abdominal reflex in the monkey other investigators have found that a series of linear pricks gives a more constant response than merely a scratch (8).

It is the belief of one clinical neurologist that the response of the normal monkey to this maneuver is more of a defensive reaction to a

nociceptive stimulus than a true superficial reflex. However, the punctate stimulus method was used throughout our examinations. In monkey C-73, like the other animals with sections of the basis pedunculi, responses on the affected side were diminished or absent.

Plantar stimulation of the left foot and palmar stimulation of the left hand evoked only a weak response, at best. The plantar response usually was absent, the palmar, diminished. Occasionally the monkey reacted to the latter maneuver as it did to a nociceptive stimulus, *i.e.*, with a generalized quick withdrawal. This reaction appears also from stimulation of the normal palm.

No value could be derived from the Hoffman's maneuver because of the variability of the responses obtained even in the normal monkey. Likewise Gonda's (5) new reflex, "a dorsal flexion of the big toe when the fourth toe of the affected hind limb is held in plantar flexion for a few seconds and then snapped with considerable force," failed to constitute a characteristic sign in the paralyzed foot.

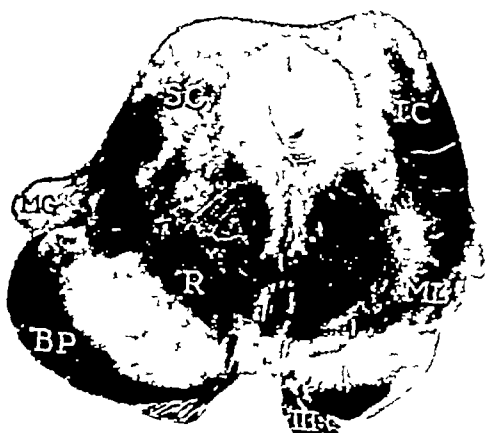


FIG. 2 Cross section of midbrain of monkey C-73 illustrating lesion in right basis pedunculi. Weil Stain.

Figure 1B depicts the same animal in the frequent and characteristic position during excitement or struggling. A semiflexion at the elbow and extension of the digits were noted in the left upper extremity. Flexion at the hip and knee and semiflexion at the ankle with foot inverted and digits rigidly extended was the characteristic position of the left lower extremity.

A constant sign of the loss of voluntary control of the affected lower extremity was noted by the authors: when the affected limb is crossed over the opposite limb it will remain in that position. Figure 1C illustrates this.

Placing and hopping reactions. In placing and hopping tests the failure to obtain response from the affected extremities was apparent in every examination. Active crossed placing and hopping responses were always carried out by the corresponding contralateral extremity, producing evidence that the fault lay within the efferent rather than the afferent system innervating the paralyzed extremities (17). The absence of visual placing with the left forelimb is illustrated in Fig. 1D. The absence of non-visual placing with the left hind limb may be seen in Fig. 1E.

Grasp. The failure of the left foot to grasp an object brought into contact with its plantar surface is shown in Fig. 1F. The normal grasping response is well illustrated by the right or normal foot. The absence of grasping was less obvious in the left hand and occasionally the digits of this hand were frozen in a position of semiflexion simulating grasping. When a weak response was elicited it seemed that relaxation time was prolonged. Usually on such an occasion the right hand gave a similar response. The failure of the normal grasp response to occur in the paralyzed limbs also became evident when climbing or walking on a horizontal bar.

Skeletal muscle tone. Following operation in monkey C-73, as in others of this series, the muscle groups of the affected hind limb were flaccid. They offered absolutely no resistance to passive motion at either the proximal or distal joints. The upper extremity was likewise affected but not as severely. The return of tone to the extensor muscle groups of the fingers and toes became evident in about two weeks. The extension of these digits was the only sign of a condition that might be considered hypertonicity. In other muscle groups little change from a hypotonic state occurred for as long as the animal was observed, which was over a year.

A transient paralysis of the lower face muscles and an ephemeral paresis of the trunk muscles on the left side was apparent following the operation. For two weeks the paralysis of the face gave all the signs of a supranuclear facial nerve lesion, *i.e.*, widening of the palpebral fissure, ironing out of the naso-labial fold and drooling from the side of the mouth. Recovery from this involvement continued until no difference between right and left sides of the face could be detected.

Reflexes. Although all tendon reflexes of the left upper and lower extremities were hyperactive, the most significant and constant sign of hyperreflexia was that of the patellar tendon reflex or knee jerk. The hyperactive response obtained by striking this tendon was accompanied by a contraction of the

Except for the case with significant injury to the medial lemniscus, in which were noticed signs of a sensory deficit, the symptoms in each of these animals were so similar as to appear identical and the findings reported for monkey C-73 are applicable to each of the other animals of this group. It is logical to attribute these symptoms to the destruction common to each of the animals, *i e*, to interruptions of the basis pedunculi and softening of the

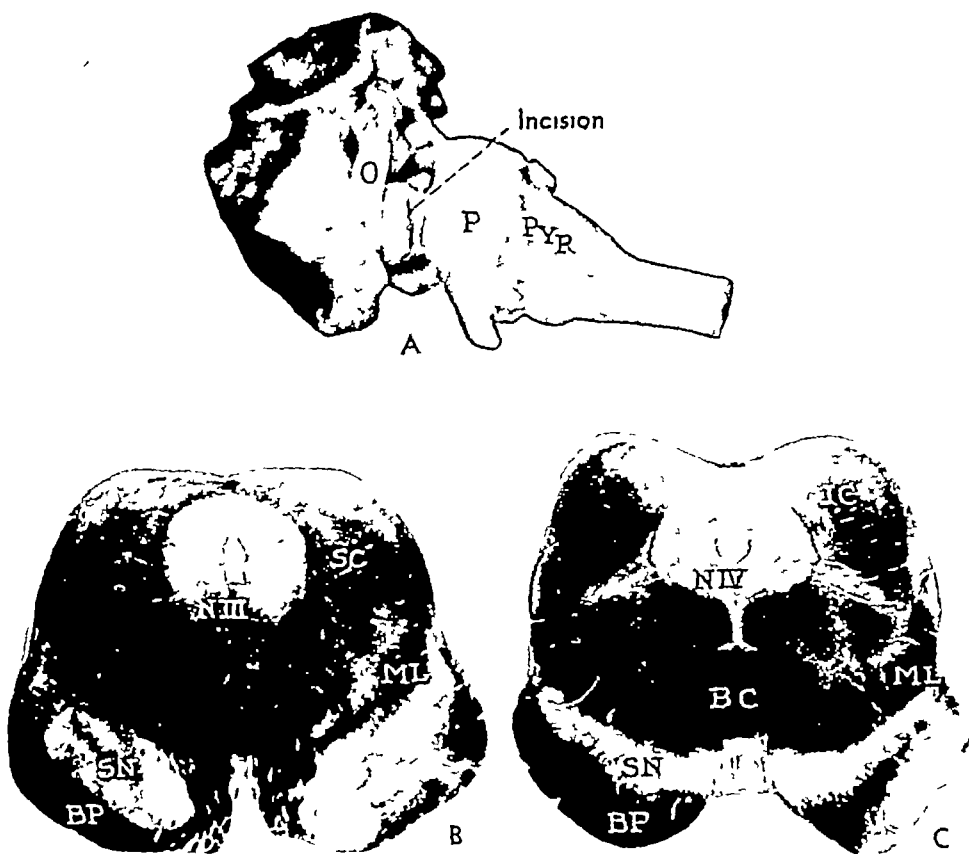


FIG. 3 Lesion of right basis pedunculi in Monkey C-88, shown in gross view (A) and in cross sections through the superior (B) and inferior (C) colliculus. Weill Stain.

substantias nigra overlying it. Ranson (unpublished data) observed that electrolytic lesions restricted to the substantia nigra are not followed by hyperreflexia and paralysis, or indeed by any clinical signs which could be detected. These observations lead us to believe that the nigral softening in the present cases was not significant in the etiology of these symptoms, which in our opinion should be attributed to interruption of the basis pedunculi.

In addition to possible complications of lesions within the brain stem, some softening of the occipital lobe of the hemisphere resulted from operative retraction or venous interruption in each case. Complete occipital lobec-

Specific performances The animal was able to use the paralyzed extremities successfully as struts when moving about although the agility with which he moved them was noticeably less than in the case of the normal extremities. The loss of specific or finer movements was brought out by an inability to clutch a wire sufficiently to support his weight when climbing. The handicap was further evidenced by the failure of the digits to assume the normal supporting posture, *i e*, fingers and toes frequently became folded under the contacting surfaces of the hand and foot.

Defensive reactions to handling were executed entirely with the right extremities. Likewise in eating, grooming, scratching, exploration and other acts the left extremities played little or no part.

Other observations Shivering and piloerection following operation apparently were confined to the left side. A drop in skin temperature was easily detected on palpation of the pads of the left hands and feet. However, gradual recovery over a period of weeks progressed until little difference in skin temperature was noted on comparing the two sides.

Atrophy of the muscles of the left limbs was apparent in about a month following the operation. Apart from the occasional manifestation of stoicism the reaction to nociceptive stimuli was equal when applied to either side.

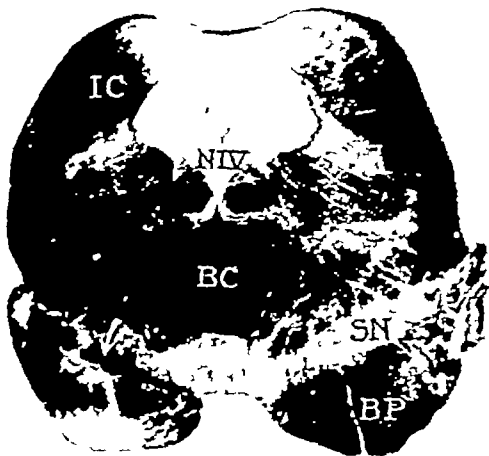
At autopsy in monkey C-73 destruction of the right basis pedunculi was verified as is shown in Fig. 2. The clinical findings in the other four animals (C-90, C-33, C-100 and C-88) were similar to those of C-73 detailed above and autopsy findings in these animals closely resembled one another. A typical lesion is illustrated in Fig. 3 from monkey C-88. In Fig. 3A the incision can be seen in the gross specimen coursing obliquely across the ventrolateral surface of the right cerebral peduncle. A rostral section through the lesion (Fig. 3B) demonstrates the most medial extent of the destruction. A caudal section (Fig. 3C) presents the most lateral destruction.

Detailed study of serial sections through the brain stem of each of these animals revealed that in the typical monkey (C-73) the lesion did not involve structures other than the basis pedunculi and the substantia nigra just above it. In monkey C-100 the nigral softening was continued dorsally as a small area of destruction involving the most ventro-medial part of the medial lemniscus and the adjacent ventro-lateral portion of the capsule of the red nucleus. In C-33, shown in Fig. 4, the dorsal extension of the softening was similar to that just described but was more widespread so that a larger part of the ventral portion of the medial lemniscus, the adjacent capsule of the red nucleus and the most ventral part of the nearby tegmentum were destroyed at the level of section. More rostrally the nigral softening extended into the lateral hypothalamic area and the medial part of the subthalamic nucleus. In C-88 the nigral softening extended dorsally rostral to Fig. 3B, to destroy the rostral part of the red nucleus in the most specific manner. In C-90 the nigral softening spread dorso-laterally to destroy the dorso-lateral tegmentum, a small part of the ventral portion of the nucleus of the inferior colliculus and, more caudally and to a slight degree, the lateral part of the brachium conjunctivum.

4s, the anterior border of area 4 and the posterior portion of area 6 were removed in each animal

The postoperative course of animal C-55, without other neural lesions, was observed for four months. Examinations on the third day following cortical ablation revealed a hypertonicity in the contralateral limbs involving primarily the flexor muscle groups, the one exception being flexors of the digits. The position of the left extremities was that of semiflexion with the digits rigidly extended and abducted. The degree of hypertonicity in the proximal muscle groups gradually became less marked, assuming normal tone at the end of about two weeks. The extension of the digits similarly

FIG 5 Cross section through inferior colliculus showing the most caudal portion of the lesion in the left basis pedunculi. In addition to medial fibers, the most dorsal or innermost fibers indicated by the arrow remained intact. Disregard the lesion on the right side, as this destruction was the result of a second operation four months later from which the animal did not recover but died during the first postoperative day.



became reduced but continued to remain apparent. Hyperactive deep reflexes, extreme at first, later became less noticeable but were always present. Results of other tests applied in routine examination were similar to those of the animals with the basis pedunculi sectioned, although responses appeared sooner and were more transient.

The course of monkey C-90, in which cortical ablation was undertaken six weeks after interrupting the basis pedunculi, was followed in the same manner. Results of tests applied on the fourth day subsequent to cortical extirpation presented a change from those recorded following basis-pedunculi section. Upon passive manipulation, hypertonus was found to have replaced the hypotonus which was evident before the second operation. The resistance to passive motion of the left lower extremity was greater on extension at the knee and flexion at the ankle. However, the resistance encountered in this limb was less than that occurring in the corresponding limb of animal C-55. The tone in the muscles of the left upper extremity was similar in quantity and distribution to that found in animal C-55. After about two weeks this increased tone had receded until the resistance to passive manipulation was relatively equal in the right and left limbs. Hyperreflexia was accentuated by the cortical destruction but other tests yielded much the same response as was recorded following the previous peduncular section.

tomy performed in a normal monkey as a control measure was not followed by any of the symptoms under consideration. Thus the presence of the occipital softening would appear to offer no complication to these results.

As mentioned previously the findings in one monkey (C-1) did not conform to the clinical syndrome established by examination of the other five animals of this series. In this exceptional case, both hypotonicity and hypoactive or pendulous tendon reflexes characterized the paralysis and the ani-

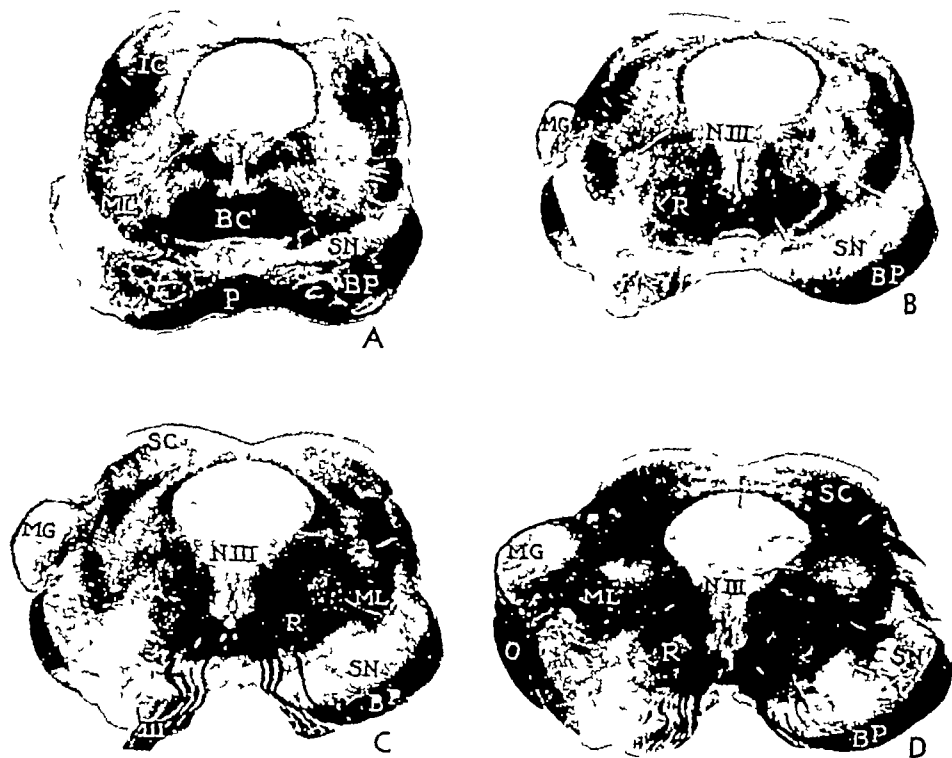


FIG. 4. Four cross sections through the midbrain of Monkey C-33, showing the lesion of the left basis pedunculi. Note softening extending into the medial lemniscus.

mal closely resembled a monkey with section of the bulbar pyramid as described by Tower (13). Study of the serial sections through the lesion in this monkey revealed that interruption of the fibers within the basis pedunculi was incomplete. In addition to a few of the most medial bundles, a rather large lemniscus of dorsal fibers remained intact as is shown on the left side in Fig. 5. These fibers diverged from the basis pedunculi and entered the brain stem tegmentum at the rostral border of the pons.

Extirpation of a portion of precentral cortex was carried out in two monkeys. One of these (C-55) was an animal without other lesions. The other (C-90) had had the right basis pedunculi sectioned six weeks before. Area

Table 1 Responses to tests following unilateral section of the basis pedunculi

	Test	Normal Side	Paralyzed Side
Lower Extremities	Passive manipulation	normal tone	hypotonus
	Clonus	absent	absent
	Patellar reflex	active	hyperactive
	Achilles reflex	active	hyperactive
	Plantar stimulation	active	absent*
	Grasp	strong	absent
	Placing reaction	present	absent
	Hopping reaction	present	absent
	Postural rigidity	absent	absent
	Palpation of muscles	firm	relatively firm
	Atrophy	absent	present
	Crossed-leg sign	absent	present
Abdomen	Abdominal reflex	active	diminished to absent
Upper Extremities	Passive manipulation	normal tone	hypotonus†
	Clonus	absent	absent
	Biceps reflex	active	hyperactive
	Triceps reflex	active	hyperactive
	Palmar stimulation	active	diminished response
	Grasp	strong	absent
	Postural rigidity	absent	absent
Face	Paralysis	absent	transient in lower‡ muscles

* One variation deserves special mention. An interesting response to plantar stimulation developed late in the postoperative course. When the Babinski maneuver was applied to this animal's affected extremity an abduction and dorsal flexion of the big toe constantly occurred.

† A slight variation from the pronounced hypotonicity was evident in the flexor and abductor muscle groups of the upper extremity in one monkey (C-88). On passive manipulation resistance to abduction at the shoulder and extension at the elbow was noted. This resistance was no greater than that exhibited in the normal forelimb of this monkey.

‡ This lasted about two weeks.

imals did not produce the classical picture of a transient spasticity as did those of Hines (7). On the contrary, the distribution of the increased tone did not reside in the extensor muscle groups of the limbs, and clonus was not a conspicuous feature. Since these observations were made, a detailed report of a spastic hemiplegia in flexion appearing subsequent to bilateral cortical ablation involving the anterior portion of area 4 and the posterior of area 6 has been published by Welch and Kennard (16). The type of paralysis they found following bilateral cortical ablation is essentially the same as we observed following unilateral ablation of the same region in animals C-90 and C-55.

DISCUSSION

If the spasticity of hemiplegia is to be attributed to the loss of cortical inhibitory influences mediated outside of the cortico-spinal tract, the organization of the neural connections involved becomes a pressing and, as yet, far from answered question. Without detracting from the importance of other observations implicating the caudate nucleus (10), the lentiform nucleus (11) and the brain stem tegmentum (12) in this regard, the present experiments have revealed an inhibitory influence mediated by connections descending in company with the cortico-spinal tract as far caudad as the basis pedunculi, for section of the basis pedunculi has been followed by markedly hyperactive tendon reflexes and exaggerated tone in the extensor muscles of the digits.

Since the symptomatology after section of the bulbar pyramid in the monkey (13) does not include either of these phenomena of release, the inhibitory connections in question must depart from the cortico-spinal tract somewhere between the midbrain and the rostral end of the medulla oblongata. Special reference may be made to the innermost lemniscus of the basis pedunculi which passes dorsolward into the tegmentum at the rostral border of the pons because there is indication of its importance in the case of monkey C-1. Here the lesion did not involve this fiber band but rather, selectively interrupted other fibers within the peduncle as is shown in Fig 5. The type of paralysis resulting in this monkey differed markedly from that seen in the other animals of this series and was more typical of that following destruction of the pyramid or of the dorso-lateral column of the spinal cord. This leads to the belief that either a sufficient number of fibers escaped injury to prevent production of the typical peduncle syndrome or that the fiber band, remaining uninjured in this case, had a rather specific inhibitory function. This evidence from a single animal is insufficient to attach a function to this lemniscus but indicates that further investigation is warranted.

In considering the information obtained from superimposing homolateral cortical ablation on a previous lesion of the basis pedunculi the results presented by one animal (C-90) offer proof that certain inhibitory fibers originating in the precentral cortex are not coursing through the peduncle and that these fibers are primarily concerned with muscle tone. Although the hyperreflexia was accentuated following the superimposed decortication, the outstanding evidence of an additional release was the development of hypertonicity which replaced the flaccidity observed subsequent to section of the basis pedunculi.

It would have been desirable to limit the cortical destruction to area 4s because the involvement of area 4 and 6 serve to complicate the picture of pure spasticity. However, since the lesion was rather extensive a similar cortical lesion was placed in a normal monkey (C-55) to furnish a control in evaluating the effect of a precentral cortical lesion in an animal with sectioned peduncle. It should be noted that the cortical lesion in these two ani-

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To return to the main topic of this report, the paralysis produced by a unilateral lesion of the basis pedunculi was intermediate between the spastic type which follows appropriate cortical extirpation and the flaccid paralysis or hypotonic paresis which follows section of the pyramid. The paralyzes in the animals of this series were spastic in that they presented hyperactive deep reflexes with hypertonicity of the extensor muscles of the digits. They resembled Tower's animals (13) with pyramidal lesions, in that hypotonicity (except for the digital muscles) and absence of clonus characterized them.

CONCLUSIONS

Interruption of the basis pedunculi in the monkey gives rise to a paralysis which is intermediate between spastic paralysis and hypotonic paresis. This paralysis is characterized by hypotonicity of all muscle groups, excepting the extensors of the digits, by hyperactive deep reflexes and by the absence of clonus.

This leads to the assumption that inhibitory pathways descending from the cerebral cortex do not course entirely within the basis pedunculi. Fibers whose interruption is responsible for the phenomenon of hypertonicity and those dealing with clonus, for the most part, have deviated from the cortico-spinal projection prior to reaching the cerebral peduncle. Those whose interruption leads to hyperreflexia accompany the cortico-spinal projection while passing through the cerebral peduncle but deviate before reaching the pyramids.

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